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THE QUANTITATIVE ESTIMATION OF SILK FIBROIN IN WEIGHTED SILK

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The industrial practise of treating silk with metallic salts in order to increase the covering power, weight, and fulness, or to change the dyeing properties came to be known as weighting because of the replacement of the weight (of from 16 to 29 per cent (44) of sericin) lost upon degumming raw-silk. This term, weighting, often applied only to the inorganic additions to silk, has been defined (60) as "all materials other than fibroin present in finished silk after it has been dried to constant weight in air at 110° Centigrade".

The first weighting agents were iron salts; later, salts of lead, tin, bismuth, copper, barium, zinc, aluminum, manganese, magnesium, chromium, antimony, tungsten, zirconium, and the rare earths were employed. Although the use of some of these salts was questionable because of toxicity, many increased the resistance of silk to flame, light, water, and micro-organisms. However, weighting with compounds of tin, first extensively employed about 1870 in France and Germany, became the principal method when the tin-phosphate-silicate process was developed by Neuhaus (39) in 1892. Silk weighted in this manner may at first have its mechanical properties slightly increased in value but upon ageing, particularly in the presence of light, acid, or salt, the mechanical properties of the silk fiber decrease in value and in extreme instances the fibrous structure is completely destroyed. The treatment of weighted silk with negative catalysts or with compounds of nitrogen or sulfur more readily oxidizable than silk fibroin, has proved of little value in the prevention or retardation of this deterioration. And yet because of its greater decorative value or its frequently lower initial cost weighted silk is to be preferred to unweighted silk for certain uses.

Although any study of weighted silk depends upon an accurate estimation of weighting, the increase in weight of a silk by the weighting process is the only accurate method for the estimation of weighting at present.

Weighting may be determined indirectly and approximately according to Serrell (49) and Testenoire (44) by counting the number of cocoon filaments and calculating the percentage of fibroin from the weight of one cocoon filament. Measurements of the electrical conductance (9) and of the specific gravity (62, 63, 50, 7, 28) of weighted silks have proved of little use in the estimation of weighting. An x-ray method, suggested by Riche (44), was recommended by Tondani (61) as rapid and accurate within five per cent. Tondani prepared a photographic negative of the silk and compared it with standards prepared from silk of known amounts of weighting.

Chemical methods for the estimation of weighting have proved better than the physical methods. The simplest of these are methods based upon a determination of the ash of weighted silk. The ash of silk fibroin prob-

ably amounts to less than one per cent (6) although higher percentages have been reported for wild silks and for degummed silks which may have contained soap from the degumming bath. Table 1 lists percentages of ash reported for silk fibroin.

TABLE 1. *The ash of silk fibroin*

Investigator	Ash	Description of silk fibroin
	percentage	
Königs (33)	0.77	scoured silk
Weyl (67)	0.56	
Knecht, Bastow, and Appleyard (32)	0.23	Tussah fibroin
Wardle and Bell (65)	0.25	
Gnehm and Dürsteler (24)	0.20	boiled-off China tram
Suzuki, Yoshimura, and Inouye (59)	0.63	<i>Bombyx mori</i> silk fibroin
Abderhalden and Spack (3)	1.67	Indian Tussah
Abderhalden and Singleton (2)	0.46	Bengal silk fibroin
Herzog and Jancke (30)	1.59	degummed silk
	0.7	Tussah fibroin
Furry (13)	2.22	degummed silk fabric
Abderhalden and Heyns (1)	0.82	Tussah fibroin

Königs (33) and Cook (7) maintained there was no definite ratio between the amount of weighting in a weighted silk and the total ash. Cook obtained ratios ranging from 1.12 to 1.56 (the factors for metastannic acid and orthostannic acid from stannic oxide and for metastannic acid and orthostannic acid from tin, respectively, are 1.1195, 1.2391, 1.4214, and 1.5731). Silbermann (51), Gianoli and Zappa (19), Sisley (54, 55), Ristenpart (47), and Weltzien (66) have, respectively, used the factors 1.13, 1.27, 1.28, 1.4, and 1.2.

The proximate analysis of weighted silk based on its nitrogen content was suggested by Sainte-Claire Deville in 1878. Moyret (36) determined the nitrogen of silk by Will and Varrentrap's method. Later the Kjeldahl method in its various modifications was used (58, 53). In table 2 are listed percentages of nitrogen reported for silk fibroin.

Various procedures were developed to remove from the silk all other nitrogenous substances. Persoz (41, 43) used soap solution and dilute alkali and Moyret (36), two or three baths of dilute sodium carbonate at 80°C. alternating with baths of very dilute hydrochloric acid. Silbermann (51) observed that boiling water and baths of 2 per cent sodium hydroxide and 2.5 per cent hydrochloric acid dissolved part of the fibroin. Steiger and Grünberg (58) prepared light-colored silks for analysis by boiling these for two hours in a 2.5 to 3.0 per cent solution of soap followed by boiling one per cent sodium hydroxide. For iron-weighted silks they used six treatments with 5 per cent hydrochloric acid at 60°C. followed by boiling 0.5 per cent sodium hydroxide for 30 minutes and boiling 2.5 or 3.0 per cent soap solution for an hour. Gnehm and Schwartz (25) showed that fibroin decreased quite appreciably in weight and in nitrogen when treated with 5 per cent hydrochloric acid. Gnehm and Blumer (23) extracted iron-weighted silks with one per cent hydrochloric acid at 60°C. until colorless, washed and treated the residue several times with 2 per cent sodium carbonate at 80°C., then with boiling 2.5 per cent soap solution for an hour and a half, and finally washed, dried, and conditioned the sample. Sisley (55) recommended as a stripping procedure a bath of 25 per cent acetic acid followed by 3 per cent disodium phosphate, and

finally by soap and sodium carbonate solutions. Steiger (57) later reported the effectiveness of 0.2 per cent hydrochloric acid as a stripping agent and Gianoli (15, 17) recommended a solution 2 per cent sodium hydroxide and 4.5 per cent glucose at 50°C. for ten minutes.

The chief objections to the method of proximate analysis have been the necessity of removing other nitrogenous substances, the time required for the analysis, and in some instances the difficulty of carrying out the analysis in a technical laboratory. However, this method is considered the most accurate for estimation of the fibroin of weighted silk.

Several procedures have been elaborated for the indirect estimation of weighting by removal methods. The first removal methods were used for iron-weighted silks. Persoz (41) recommended prolonged boiling with potassium acid oxalate or repeated treatments with dilute oxalic acid alternating with dilute sodium carbonate. Müller (38) used saturated oxalic acid at room temperature but this was found unsatisfactory by Gnehm and Dürsteler (24) who employed 3 to 5 per cent oxalic acid and 2 per cent sodium carbonate. Persoz (41) also recommended repeated treatment with dilute hydrochloric acid alternating with sodium carbonate. Martinon (44) used 1:3 hydrochloric acid and 2.5 per cent sodium hydroxide. Ristenpart (46, 47) noted that 10 per cent hydrochloric acid removed only the inorganic weighting and suggested that an alkali, preferably N potassium hydroxide, be used to dissolve the organic finishing substances. Heermann (27, 29) considered this treatment too severe for the silk and advised instead the use of equal volumes of glycerol and N potassium hydroxide at 80°C. Another procedure developed by Persoz (44) was the use of a warm bath of dilute ammonium hydroxide followed by alternate baths of 1:3 hydrochloric acid and 2.5 per cent sodium hydroxide at room

TABLE 2. *The total nitrogen of silk fibroin*

Investigator	Nitrogen	Description of silk fibroin
	percentage	
Mulder (37)	17.60	
Städeler (56)	18.66, 18.69, 19.33	
Cramer (8)	18.21, 18.36, 18.40	
Bolley and Schoch (4)	18.50, 18.78, 19.40	
Schützenberger and Bourgeois (48)	18.7	
Moyret (36)	17.6	at 10 per cent humidity
Persoz (42)	18.0	air-dry
Weyl (67)	17.63, 17.87	
Knecht, Bastow, and Appleyard (32)	16.80, 16.90	Tussah fibroin
Vignon (64)	19.2	
Filsinger (11)	14.34, 14.45	Tussah
	16.00, 16.45	mulberry
Steiger and Grünberg (58)	18.30, 18.35	
Gerhardt (55)	17.35	
Sisley (55)	18.38	
Suzuki, Yoshimura, and Inouye (59)	18.98	<i>Bombyx mori</i>
	18.87	Sakusan
	17.73	Yamamai
	16.73	Kuriwata
Brigl and Held (5)	19.20	
Furry (13)	18.46	degummed silk fabric
Forbes and Mack (12)	18.68	degummed silk fabric
Abderhalden and Heyns (1)	17.57	Tussah fibroin
Jones (31)	18.00	
Ongaro (40)	15.89	

temperature. Alkaline sulfides were added to the alkaline baths, the silk was rinsed after each bath, and the procedure was repeated until the extracts were nearly colorless when the residues were treated with 1:3 hydrogen peroxide at 60°C., washed, heated for 15 minutes at 60°C. in very dilute hydrochloric acid, rinsed, and dried. Gnehm and Dürsteler (24) placed a sample of one gram in hot one per cent hydrochloric acid through which hydrogen sulfide was passed for 30 minutes at from 70° to 80°C. The silk was then washed three times in cold water, immersed in 4 per cent sodium hydrogen sulfide for 5 minutes at 40° to 50°C. and for 15 minutes in 2 per cent sodium carbonate at 60° to 70°C., and after half an hour in hot water the residue was dried and weighed.

In 1903 removal methods especially developed for tin-phosphate-silicate weighting were published by Gnehm and Weber (22, 26), Müller (38), and Zell (68). Gnehm and Weber used 2 per cent hydrofluoric acid for one hour at room temperature and obtained less than one per cent loss in weight of the fibroin and a residue of between 0.6 and 1.29 per cent ash. Müller employed dilute hydrofluoric acid, 0.4 or 1.0 per cent, at room temperature and Ris (45) showed that the residual ash was less than 0.3 per cent by this method. Zell made use of 1.5 per cent hydrofluoric acid at from 50° to 60°C. for about 20 minutes. Gnehm and Dürsteler showed that two extractions for 15 minutes each with one per cent hydrofluoric acid were enough to decrease the residual ash to 0.08 per cent. Ristenpart (46) reported the use of 10 per cent hydrofluoric acid for an hour. Gianoli (14, 18, 55) showed that upon treatment with one per cent hydrofluoric acid for 20 minutes at from 50° to 60°C. the residual ash varied as the amount of weighting and later (16) that this method was reliable only for tin-weighting fixed with sodium silicate. Farrell and Goldsmith (10) reported that repeated treatments with hydrofluoric acid and sodium carbonate affected the fibroin although silk boiled three times with sodium carbonate suffered no loss of nitrogen. Heermann and Frederking (29) observed that boiling 2 per cent hydrofluoric acid dissolved 0.2, 0.8, and 1.8 per cent of fibroin in 15, 30, and 60 minutes respectively. Cook (7) found that fibroin was attacked by 2 per cent hydrofluoric acid in 5 minutes boiling as recommended by Matthews (35). Weltzien (66) used 7 per cent hydrofluoric acid at room temperature according to Stüssi.

Hydrofluosilicic acid was recommended as a substitute for hydrofluoric acid (20) but was later (26) shown about twice as destructive and to remove (21) only about 20 to 65 per cent of the weighting.

The methods for the determination of weighting developed by the Bureau of Standards (60) are as follows:

“IV. Tin Phosphate Silicate Weighted Silk.—1. The sample is dried to constant weight in an air oven at 100°C. This is called weight A.

2. The dried sample is soaked in 100 times its weight of distilled water at 65° C. for 20 minutes. It is moved about in the water every few minutes during this time in order to insure thorough penetration of water and extraction of water-soluble materials. It is then rinsed in a fresh portion of distilled water, then in alcohol, and finally in ether, after which it is dried to constant weight as above. (Two 25 cc. portions of alcohol and of ether are usually sufficient.) This is called weight B.

Weight A — weight B \times 100/weight A = finishing materials in per cent

3. The sample from which “finishing material” has been removed is soaked in 100 times its weight of 2 per cent hydrofluoric acid solution

at 65°C. for 20 minutes. It is then rinsed in water and treated in 100 times its weight of 2 per cent sodium carbonate solution at 65°C. for 20 minutes. It is then rinsed in water, in alcohol, and in ether and dried to constant weight as before. This is called weight C.

4. The sample is then ashed and the ash weighed, weight D. This ash should not weigh more than one-tenth of the difference between weight B and weight C. If the silk contains a considerable amount of weighting, the treatment given in (3) above should be repeated with fresh solutions before the sample is ashed in order to obtain a low ash. A few threads of the silk treated along with the sample and ashed will show the operator whether the weighting has been removed in (3) or if the treatment must be repeated.

5.
$$\frac{\text{Weight A} - \text{weight C} + \text{weighted D} \times 100}{\text{Weight A}} = \text{weighting in per cent.}$$

“V. Tin Phosphate Weighted Silk.—1. The procedure for silk which does not contain silicate is the same as that given in Section IV above, except that the following will be substituted for paragraph 3: The sample from which finishing material has been removed is soaked in 100 times its weight of 4 per cent hydrochloric acid solution at 55°C. for 20 minutes. This is repeated with a fresh solution. The sample is then rinsed in water and soaked in 100 times its weight of 10 per cent sodium carbonate solution at 55°C. for 20 minutes. It is then rinsed in water and the hydrochloric acid treatment repeated. The sample is again rinsed in water, then in alcohol and in ether, and dried to constant weight as before. This is called weight C.

“VI. Logwood Black Weighted Silk and Silk Weighted with Lead, Zinc or Aluminum Salts.—1. If silicate is present, the procedure is the same as that given in Section IV, except that the treatment with hydrofluoric acid is preceded by a treatment with 100 times the weight of the sample of 4 per cent hydrochloric acid solution at 55°C. for 20 minutes repeated once with a fresh portion of the solution.

2. If silicate is not present, the procedure is the same as that given in Section V.”

EXPERIMENTAL

The silks studied were plain-weave constructions of degummed silk, white tin-phosphate-weighted silk, and white tin-phosphate-silicate-weighted silk. The tin-phosphate-weighted silk was treated according to the Bureau of Standards (60) method V, the tin-phosphate-silicate-weighted silk according to method IV, and the degummed silk according to each of these methods (the procedure in paragraph 4 was carried out but once). Samples were dried to constant weight at 105° to 110°C. and weighings were made with tares. The solutions were brought to the required temperatures in Erlenmeyer flasks (500 cc.) before the silks were introduced. The temperatures were maintained at 55° or 65°C. ($\pm 0.1^\circ\text{C.}$) in a DeKhotinsky water bath. After each treatment the samples were dried to constant weight; a number were ashed to constant weight in an electric furnace at dull red heat and a number were analyzed for nitrogen by the Kjeldahl method. The data are given in tables 3, 4, and 5.

TABLE 3. *Unweighted silk*

Sample	Weight	Residue from treatment with water	Residue from treatment with hydrofluoric acid	Residue from treatment with hydrochloric acid	Nitrogen of last residue	Ash of last residue
number	grams	percentage	percentage	percentage	percentage of sample	percentage of sample
1	3.6633				18.50	
2	3.8388				18.50	
3	3.6435				18.54	
4	3.6126				18.55	
average					18.52	
5	5.0357					0.33
6	4.8644					0.34
7	4.9125					0.35
8	4.5540					0.36
average						0.35
9	2.8865	99.74			18.40	
10	2.5169	100.10			18.51	
11	3.4559	99.73			18.55	
12	3.4030	99.75			18.59	
average					18.51	
13	3.4498	99.70	100.16		18.40	
14	3.4823	99.80	99.65		18.45	
15	3.6558	99.83	100.04		18.54	
16	3.4080	99.72	99.76		18.62	
average			99.90		18.50	
17	3.5845	99.23		98.34	18.39	
18	3.6805	99.61		98.10	18.49	
19	3.7786	99.72		98.70	18.54	
20	3.6498	99.59		98.63	18.59	
average		99.71		98.44	18.50	

TABLE 4. *Tin-phosphate-weighted silk*

Sample	Weight	Residue from treatment with water	Residue from treatment with hydrofluoric acid	Nitrogen of last residue	Ash of last residue
number	grams	percentage	percentage	percentage of sample	percentage of sample
1	5.4012			10.95	
2	5.1802			10.97	
3	5.3562			11.04	
4	5.2996			11.13	
average				11.02	
5	5.6383				35.71
6	5.1032				35.80
7	5.5072				35.89
8	5.1282				35.96
average					35.84
9	3.9040	97.84		10.57	
10	3.8719	98.01		10.62	
11	4.0111	97.86		10.68	

TABLE 4. (Continued)

12	4.0410	97.92		10.70	
average				10.64	
13	4.0589	97.91			34.62
14	3.9096	98.04			34.70
15	4.0039	98.02			34.82
16	4.1374	98.18			34.84
average					34.75
17	3.9059	98.47	60.33	10.48	
18	4.0208	97.74	60.72	10.64	
19	3.9116	98.32	61.39	10.74	
20	4.0758	98.26	60.83	10.77	
average				10.66	
21	4.1024	98.37	60.39		2.43
22	3.8974	98.13	60.62		2.55
23	4.0810	98.33	60.94		2.69
24	4.1012	98.37	61.11		2.69
average		98.11	60.79		2.59

TABLE 5. *Tin-phosphate-silicate-weighted silk*

Sample	Weight	Residue from treatment with water	Residue from treatment with hydrofluoric acid	Nitrogen of last residue	Ash of last residue
number	grams	percentage	percentage	percentage of sample	percentage of sample
1	6.1668			7.71	
2	6.6531			7.74	
3	6.4120			7.75	
4	6.4472			7.78	
average				7.75	
5	6.2971				46.74
6	6.0796				46.83
7	6.3658				46.94
8	6.0800				46.99
average					46.88
9	4.3225	91.78		7.54	
10	4.1443	91.51		7.55	
11	4.0375	91.90		7.58	
12	4.2473	91.94		7.58	
average				7.56	
13	4.1640	92.37			45.43
14	4.0681	92.25			45.46
15	4.0669	92.13			45.50
16	4.1133	92.34			45.53
average					45.48
17	4.4482	92.15	44.11	7.50	
18	4.5013	91.95	44.31	7.53	
19	4.3047	92.42	43.79	7.56	
20	4.1360	91.20	44.79	7.57	
average				7.54	
21	4.1618	92.01	45.15		0.27
22	4.1365	91.77	44.82		0.28
23	4.4311	91.62	44.56		0.30
24	4.2158	92.02	44.29		0.30
average		91.96	44.47		0.29

The percentages of fibroin calculated from the nitrogen, the ash, and by the removal methods are given in table 6.

TABLE 6. *Percentage of fibroin in weighted silks*

Silk	Basis of calculation			
	Nitrogen	Removal methods	Ash	Ash x 1.1195
Tin-phosphate-weighted (before treatment with water)	59.51	58.20	64.51	60.27
Tin-phosphate-weighted (after treatment with water)	57.46			
Tin-phosphate-silicate-weighted (before treatment with water)	41.85	44.18	53.47	47.91
Tin-phosphate-silicate-weighted (after treatment with water)	40.82			

If fibroin is the source of the nitrogen lost by the weighted silks upon treatment with water, it would seem that weighting makes the fibroin less stable to water and there arises the question of the loss of nitrogen in wet finishing processes for weighted silks.

SUMMARY

1. The steps in the procedures of the standard methods for the removal of weighting from tin-phosphate-weighted silk and from tin-phosphate-silicate-weighted silk have been followed by determinations of weight, nitrogen, and ash.
2. The weighted silks were found to lose an appreciable amount of nitrogen when treated with water at 65°C. for 20 minutes but no nitrogen by the acid and alkaline treatments.

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TECHNIQUE FOR THE GROWTH OF LEMNA UNDER STERILE CONDITIONS WITH CONTROLLED TEMPERATURE AND LIGHT

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The successful growth of Lemna under sterile conditions was announced at the Des Moines meeting of the American Association for the Advancement of Science in December 1929. It was reported in *Science*¹ and the details were published² in 1931. Sterilization of *Lemna major* was also reported later by Saeger,³ and of *Lemna minor* by Hopkins.⁴

The technique developed at Iowa State College for the transfer of the plants has been very successful. During the past year subcultures were made on 15 to 30 flasks, twice a week, with but a single contamination.

The plants are grown in 100 cc. culture media contained in 250 cc. Pyrex Erlenmeyer flasks. The mouth of the flask is closed with the usual cotton stopper, the lower part of which is wrapped with cheesecloth to prevent unwinding. Such stoppers are usable until the top is burned almost to the glass.

The transfer chamber. In order to subculture, or to place all the plants in fresh medium, use is made of a small chamber of galvanized iron, two by one and one-half feet by two and one-third feet high—figure 1. The top two thirds of the front wall is made of glass and below the glass two circular holes are cut for the hands for manipulation. These holes, sufficiently large to admit an Erlenmeyer flask, are closed with hinged discs when the chamber is not in use. In the right corner at the rear there is a recess in which stands a Bunsen burner with pilot light—the burner being outside the chamber with the tip protruding through a small hole in the top of the recess. An extension handle through the wall enables the flame to be turned off except at the time of flaming the flasks; the pilot light only burning continuously during the changing. This method keeps the chamber much cooler than it would be with the full flame. A copper chimney is provided, with an inlet from the outside for draught about half way up. There is ample room between the top of the burner and the bottom of the chimney to flame flasks.

In the center of the roof a closed copper tube is inserted. This has pinholes on the circumference through which sterile air can be forced. Compressed air is first passed through a red hot coil of copper and then through a similar coil which is water cooled. The air is further filtered through a large flask filled with cotton wool and led to the tube in the roof. Before starting the first change the whole of the flask, coils etc., are sterilized

¹ Clark, Norman. "Auximones" and the stimulation of Lemna by organic matter. (1930) *Science* 71: 268-9.

² Clark, Norman and Roller, E. M. The stimulation of Lemna major by organic matter under sterile and non-sterile conditions, (1931) *Soil Sci.* 31: 299-309.

³ Saeger, A. A method of obtaining pure cultures of *Spirodela polyrrhiza*. (1930) *Bul. Torrey Bot. Club.* 57: 117-122.

⁴ Hopkins, E. F. Manganese and the growth of *Lemna minor*. 1931. *Science* 74: 551-2.

half an hour in steam at 20 lbs. pressure; this needs occasional repetition. A constant stream of sterile air is thus passing from the top downwards. Agar plates exposed one minute at the beginning and in the middle of a series of changes showed no bacterial or mold colonies.

Before the changing is started a fine fog of saturated phenol solution is sprayed from an atomizer through one of the holes in the front, and this is allowed to settle for about two hours. The atomizer was made from a liter Erlenmeyer as shown in figure 2, and is run by compressed air.

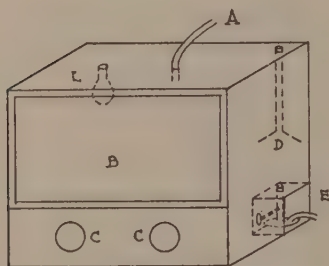


Fig. 1. TRANSFER CHAMBER: A sterile air; B glass front; C openings for hands; D chimney; E recess and burner; L light.

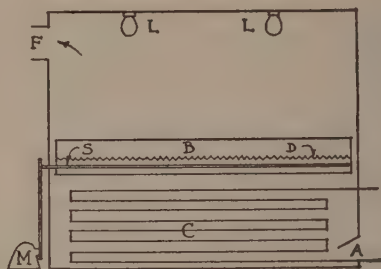


Fig. 3. GROWTH CHAMBER: A air entrance; B water bath with heating coils; C cooling coils for chamber; D wire gauze; F fan; LL lights; M motor, S stirrer.

After the sterile air has run through the chamber for a few minutes the arms and hands of the operator are dampened with a wet towel; the flasks, also dampened, are passed through the holes in the front and placed in a mold of plaster of Paris so fashioned that the flasks are in a partly reclining position, but with the solution not touching the stopper. The stoppers are flamed and put out by covering with a 50 cc. beaker, the flasks replaced in the molds and the stoppers removed by the left hand. The right hand flames a bent platinum wire with glass handle, and the transfer is made. It is convenient to have a hook on the right side of the chamber on which to hang the platinum wire. Transfers can be made at the rate of one flask every two minutes.

The growth chamber. The plants are grown in a double walled wooden chamber four and one-half by three feet by five and one-fourth high (fig. 3) containing a water bath (with cooling and heating coils) which is placed about half way from the bottom. Below the bath is a series of pipes through which cold water is run. Air is drawn around these pipes and over the bath by an exhaust fan near the top of the chamber. This gives a constant supply of cold air, and is used instead of water cooling the lights. There are four lamps, 300 watt, in the roof, so spaced as to give a very uniform illumination at the bath surface. The walls and roof of the chamber are painted white, the bottom of the bath is black.

The water bath is six inches deep, with a wire gauze half way from the bottom; below the gauze are the heating coils, the cooling coils through which cold water is turned automatically, and a stirrer. The flasks rest on the gauze with the water at the same level as the solution inside the flasks.

As the lights are burning about 15 hours a day only, there is a somewhat wide variation in temperature to be controlled. To keep to $\pm 0.5^{\circ}\text{C}$. a small ether-mercury control has been found satisfactory. This control

cuts off the heating elements (and the fan during the time the lights are off) and at the same time turns on the water. The variation is usually not much greater than 0.2° on a Beckman thermometer.

The ether-mercury control (fig. 4) is a small bore glass tube with a bulb blown at one end. The bulb is partly filled with ether and mercury and a platinum contact is sealed through the side. At the open end a tungsten wire contact is inserted; this can be raised or lowered as required and is kept in place by a screw at the top.⁵



Fig. 4. ETHER-MERCURY CONTACT: *A* vapor; *B* ether; *C* mercury.

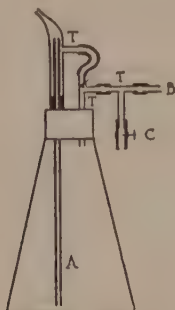


Fig. 2. PHENOL SPRAY: *A* capillary tube; *B* compressed air entrance; *C* screw clamp to regulate pressure; *T* T-tubes.

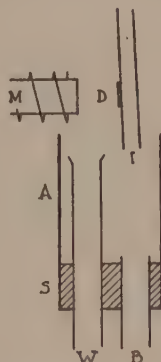


Fig. 6. WATER CUT-OFF: *A* large glass tube; *B* to coils in water bath; *D* iron disc; *I* inlet for water; *M* magnet; *S* stopper; *W* to waste.

The contact (C_1 , fig. 5) is connected with a current from an American Instrument Co. rectifying transformer (r.t., fig. 5) and to a midget relay (M.R.) of the Struthers Dunn Co. which operates on two watts (6 volts). This gives a very small spark at the contact C_1 and causes little fouling at the mercury surface. When the mercury rises and contact is made the midget relay closes a second circuit from the 110 volts A.C. line through a 75 watt carbon lamp. This second circuit excites the magnet in a Struthers Dunn Co. relay with tilting mercury content (R_1) which cuts off the main heating current. The fan (*F*) is also on this main current during the time the lights are off; the cold air is thus drawn over the bath only when it is heating.

The main circuit, on which are the bath heating coils (*H*), operates a bell transformer (*B*) which is connected with a coil and magnet. The magnet acts on an iron disc fastened to a movable tube in which is running water (fig. 6). When the bath is heating the tube is pulled to one side and the water goes to waste. As the current is cut off the heating circuit and leaves the magnet (*M*), the tube moves back, sending the water through the cooling coils in the bath. If the electricity should fail the water would keep the temperature low by flowing through the cooling coils. A further precaution is a second ether-mercury control (C_2) connected directly with a mercury relay (R_2) placed in the heating circuit. Ordinarily this mercury relay is down and the current flows, regulated by the midget relay and the first main mercury relay (R_1); if the temperature rises through

⁵ Ostwald-Luther. *Physico-Chemische Messungen*. Af. 3. Leipzig p. 111.

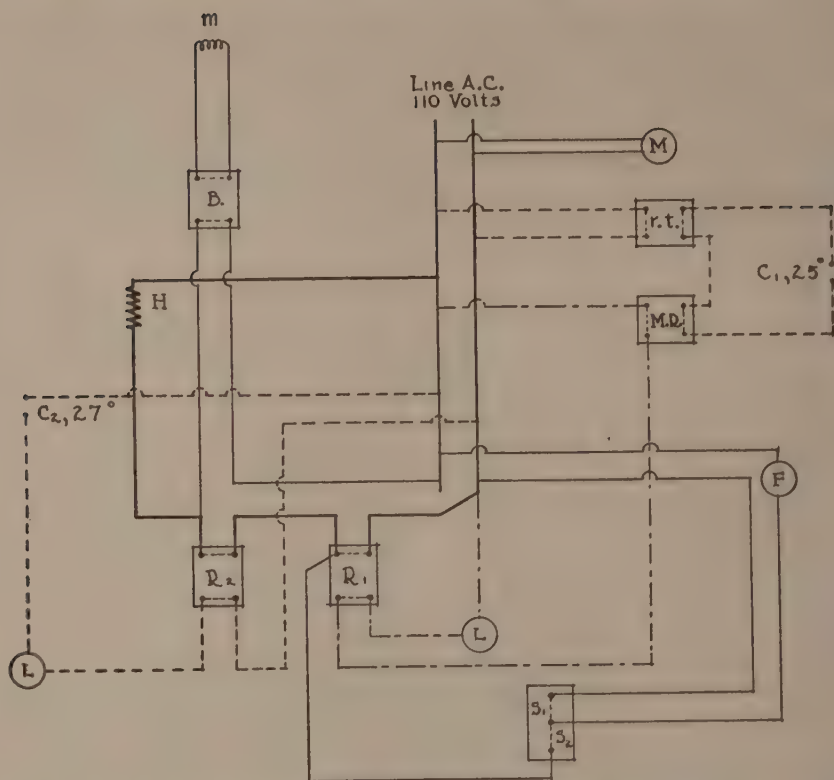


Fig. 5. WIRING FOR GROWTH CHAMBER: *B* Bell transformer; *C*₁ contact in bath 25°; *C*₂ contact in bath 27°; *F* fan; *H* Heaters in water bath; *L* Lamps 75 watts; *M* motor for stirrer; *MR* midget relay; *m* magnet for water; *R*₁, *R*₂ mercury contact relays; *r.t.* rectifying transformer; *S*₁ switch, closed when lights on; *S*₂ switch, closed when lights off.

some failure in the main circuit, the second control functions at about 27°C. causing the mercury relay (*R*₂) to break the current. Up to the present this relay has not been called upon to function. A low temperature, while it might spoil the experiment, would not kill the plants, while a high temperature would destroy them and sterilization would have to be started over again.

Although this chamber is not altogether automatic, it requires comparatively little attention. The lights are switched on (with the fan) in the evening and allowed to run until morning. The water in the bath is made up at that time as a certain amount is lost by evaporation.

Investigations are in progress on the growth of *Lemna* with reference to the effect of manganese, of iron and of organic matter, including humic acids.

SUMMARY

1. The technique employed for the transfer of *Lemna* under sterile conditions is given.
2. The growth chamber, with light and temperature controlled, and the necessary wiring are described.

THE PARTITION OF THE CONSTITUENTS OF THE CORNSTALK BY THE ACTION OF ALKALI

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In a preceding publication (1), analytical data was presented to show that the mature cornstalk consisted mainly of the three classes of compounds: pentosan bodies, lignin bodies and cellulose. The industrial process for pulping the cornstalk (2), like the industrial processes for the other grasses, is of the alkali type. In this process, the pentosan and lignin bodies removed by the caustic are sacrificed for the more valuable alkali used in the treatment. The following study reports the partition of the three predominant classes of compounds of the cornstalk under alkali treatment; the object of the study being to obtain data bearing on the feasibility of the fermentation of the pentosans in the alkali liquor.

SEPARATION OF TISSUE

The material used in these studies consisted of the two distinct types of structural tissue in the cornstalk; the pith (parenchymatous tissue), and the outer shell together with the fibrovascular bundles from the central portions of the stalk. These tissues were separated by first chopping the stalk, freed from leaves and dirt, in a Wiley mill without screens. When the mill is run at low speed (240 r.p.m.), this will break the stalks into pieces one to two inches in length and crush the outer shell of all pieces. The cubical pith cells are then screened from the fibrovascular bundles and outer shell by the use of a wash roll in an ordinary beating engine; the beater roll being set with at least one-fourth inch clearance and the wash roll being covered with a 10 mesh screen. Concentration of wash water and prevention of water waste can be obtained by returning the water from the wash roll to the beater after filtering out the pith by means of 60 mesh screen.¹ The separation is completed in about 45 minutes with the experimental apparatus used in this laboratory. The average of seven such separations gave the following results:

Pith material	26 per cent
Fibrous material	63 per cent
Water soluble material	9.0 per cent
Loss (by diff.)	2.0 per cent

It should be emphasized that the separation of these tissues is made without chemical treatment of the stalk, and (while a Hollander is used in the separation) that the tissue is not "beaten" in the usual sense of the word. Many other kinds of washing machines could be developed for the same purpose, the fundamentals involved being merely the rubbing out of the soft pith and the separation of these cubical cells from the more fibrous and resistant tissue. This separation, if used commercially, would take

¹ A patent covering the essentials of this apparatus has been issued to Kumagata and Shimomura, B. P. 299740, Chem. Abs. 23, 3574, 1929.

the place of the hot water washing shown in the flow-sheet for the commercial preparation of paper from cornstalks (2).

PULPING OF TISSUE

The chemical treatment reported for the two tissues of the cornstalk consists essentially of the de Vains process. The literature regarding the de Vains process is limited primarily to patents² and to descriptions of plant operation (3). Mutti and Venturi (4) report an analytical study of the process on straw. A number of studies have been made in the Chemical Engineering Department of Iowa State College on the application of this process to the production of cellulose from the cornstalk (5).

The de Vains process consists of three distinct steps:

1. Preliminary treatment with dilute alkali to partially delignify the tissues.
2. Chlorination of the pulp from the alkali treatment to convert the residual lignin into soluble compounds.
3. Extraction of the chlorinated lignins with either sodium sulfite or caustic solutions.

It was expected that each of these treatments would cause sufficient chemical change that a three dimensional diagram would be necessary to portray the results graphically. Accordingly, three variations in each treatment were made giving a total of twenty-seven samples for the study. The expected variation in the second and third treatments was not found, the greater portion of the chemical change taking place in the first treatment with dilute caustic. While the second and third treatment caused little chemical change, they did cause considerable change in the case of bleaching of the pulp.

In applying these treatments to the cornstalk tissues, quantitative procedures were followed throughout rather than pulping processes. The tissue, excepting the pith, was ground to pass a 60 mesh screen. The material used had a moisture content of 5.63 per cent on an oven-dry basis of 105 degrees. The dry tissue ran 1.44 per cent ash. All results reported are on the oven-dry basis.

In the study of the pulping of the fibrous material, twenty-seven samples of twenty-five grams each were weighed out to give three variable treatments of each of the three steps in the procedure. These samples were then grouped as shown in table 1 to give all the possible combinations of:

1. Alkali treatment with concentrations of 0.5, 1.0 and 2.0 volume per cent sodium hydroxide.

² de Vains

U. S. Patent	1,106,994
U. S. Patent	1,500,060
U. S. Patent	1,556,497
U. S. Patent	1,593,487
British Patent	189,561
British Patent	197,329
British Patent	198,975
British Patent	201,488
British Patent	208,551

TABLE 1. *Results of various treatments on fibrous material*

Sample No.	Treatment				ANALYSIS OF LIQUORS						Yield of pulp	Analysis of Pulp				Combustible matter + yield of pulp	Pentosan removed + pento-	Total solids + water soluble material
	Conc. Na ₂ SO ₄ percentage	Vol. NaOH cc.	Chlorination time mins.	Conc. Na ₂ SO ₄ percentage	NaOH			Chlorination				Ash	Pentosan	Lignin	α cellulose			
					Combustible residue	Pentosan	Combustible residue	Pentosan	Combustible residue	Pentosan								
1	0.5	250	15	1.0	14.64	1.58	1.53	0.15	2.38	—	18.55	0.25	22.25	13.62	52.00	90.75	—	99.95
2	0.5	250	15	2.0	14.64	1.58	1.53	0.15	4.01	—	20.18	0.29	21.12	13.47	50.32	91.18	—	100.38
3	0.5	250	15	4.0	14.64	1.58	1.53	0.15	6.68	—	23.85	0.25	19.51	12.86	47.87	92.25	—	101.45
4	0.5	250	30	1.0	14.64	1.58	1.77	0.16	2.39	0.71	18.80	0.32	18.90	11.19	49.38	86.80	21.35	96.00
5	0.5	250	30	2.0	14.64	1.58	1.77	0.16	2.26	0.75	18.67	0.26	18.41	10.79	47.45	85.17	20.90	94.37
6	0.5	250	30	4.0	14.64	1.58	1.77	0.16	2.27	0.65	18.66	0.21	19.17	10.52	48.98	85.36	21.56	96.16
7	0.5	250	60	1.0	14.64	1.58	2.41	0.17	6.71	0.89	23.76	0.29	17.86	10.40	44.00	86.86	20.50	96.06
8	0.5	250	60	2.0	14.64	1.58	2.41	0.17	5.11	1.26	22.16	0.30	18.94	7.04	43.18	84.96	21.95	94.16
9	0.5	250	60	4.0	14.64	1.58	2.41	0.17	4.63	1.18	21.68	0.26	18.55	7.58	44.35	84.78	21.48	93.98
10	1.0	250	15	1.0	24.75	3.61	1.41	0.24	1.93	0.58	27.09	0.15	17.29	4.32	42.02	84.19	22.72	93.39
11	1.0	250	15	2.0	24.75	3.61	1.41	0.24	—	0.42	—	0.13	17.80	4.57	42.40	—	22.07	—
12	1.0	250	15	4.0	24.75	3.61	1.41	0.24	—	0.42	—	0.11	16.96	4.84	39.40	—	21.23	—
13	1.0	250	30	1.0	24.75	3.61	1.60	0.34	2.14	0.72	28.49	0.10	17.45	3.41	40.20	84.49	22.12	93.69
14	1.0	250	30	2.0	24.75	3.61	1.60	0.34	2.30	0.69	28.65	0.13	17.90	3.12	40.60	84.30	22.54	93.50
15	1.0	250	30	4.0	24.75	3.61	1.60	0.34	2.14	0.54	28.49	0.10	17.95	3.24	40.45	84.99	22.44	94.19
16	1.0	250	60	1.0	24.75	3.61	1.64	0.30	2.41	0.87	28.80	0.16	17.67	3.34	39.35	84.90	22.45	94.10
17	1.0	250	60	2.0	24.75	3.61	1.64	0.30	2.58	0.95	28.97	0.14	17.05	2.92	38.76	84.07	21.91	93.27
18	1.0	250	60	4.0	24.75	3.61	1.64	0.30	2.58	0.84	28.97	0.18	16.50	2.84	38.30	83.77	21.25	92.97
19	2.0	250	15	1.0	30.20	7.53	1.20	0.12	3.42	0.44	34.82	0.21	15.25	3.02	37.00	88.02	23.34	97.22
20	2.0	250	15	2.0	30.20	7.53	1.20	0.12	2.82	0.39	34.22	0.16	15.30	3.18	41.00	89.32	23.34	98.52
21	2.0	250	15	4.0	30.20	7.53	1.20	0.12	2.20	0.30	33.60	0.14	18.30	3.54	43.75	91.10	26.25	100.30
22	2.0	250	30	1.0	30.20	7.53	1.46	0.20	2.11	0.67	33.77	0.14	14.60	2.26	38.90	86.58	23.00	95.78
23	2.0	250	30	2.0	30.20	7.53	1.46	0.20	1.78	0.60	33.44	0.15	14.39	2.16	38.20	85.24	22.72	94.44
24	2.0	250	30	4.0	30.20	7.53	1.46	0.20	2.73	0.63	34.39	0.17	14.07	2.18	37.60	86.19	22.43	95.39
25	2.0	250	60	1.0	30.20	7.53	1.84	0.17	2.22	0.92	34.26	0.27	14.82	2.53	37.85	86.36	23.44	95.56
26	2.0	250	60	2.0	30.20	7.53	1.84	0.17	1.97	0.78	34.01	0.25	14.38	2.36	38.90	85.91	23.86	95.11
27	2.0	250	60	4.0	30.20	7.53	1.84	0.17	1.44	0.73	33.48	0.25	13.70	2.39	38.28	84.38	22.13	93.58

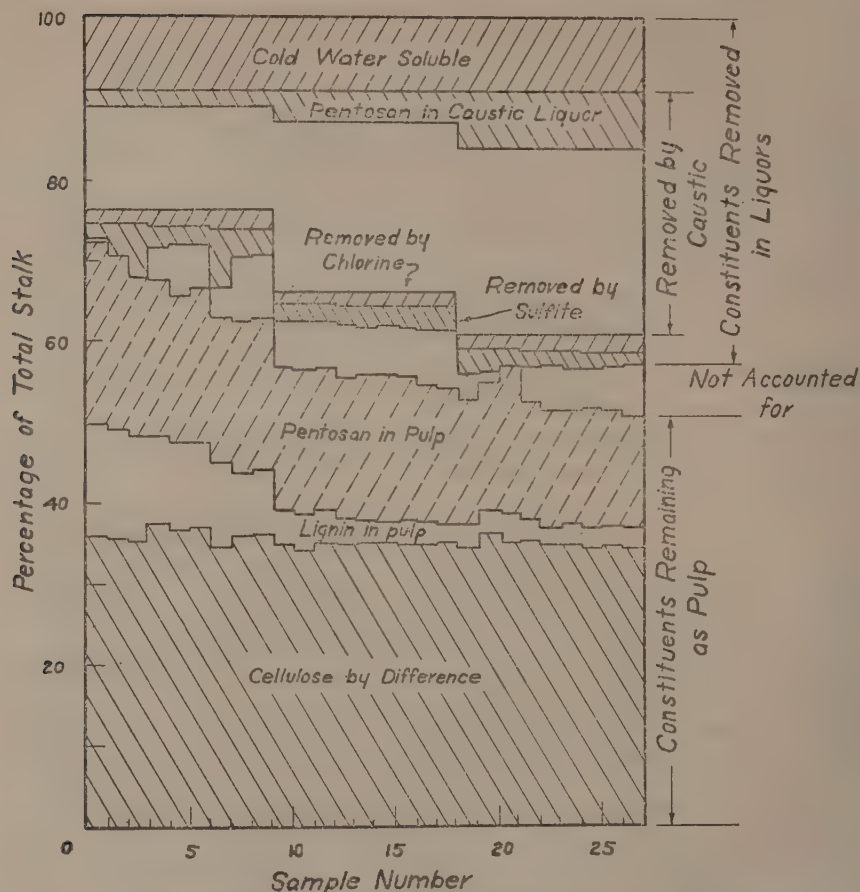


Fig. 1. Diagram of partition of fibrous material with various treatments from data of table 1.

2. Chlorination with chlorine gas at a constant rate of 100 cc. per minute for 15, 30 and 60 minutes.
3. Treatment with 1, 2 and 4 per cent sodium sulfite.

The exact pulping procedure is given in detail below. The results are tabulated in table 1 and are shown graphically in figure 1.

The results for the pulping of the pith are tabulated in table 2. The pulping of the pith was carried out in exactly the same manner as the pulping of the fibrous material except 750 cc. caustic solution were used for each 25 grams of tissue. This increase in volume of caustic is necessitated by the bulky nature of the pith.

LABORATORY PROCEDURE

Twenty-five gram samples were used, the smallest volume of sodium hydroxide of the indicated concentration sufficient to cover the sample was

TABLE 2. *Effect of caustic concentration on yield of pentosan*

Sample No.	0.1	0.2	0.3	0.5	1.0	2.0	3.0	5.0	10.0	15.0	20.0	25.0	30.0	35.0	40.0	45.0	50.0	55.0	60.0	65.0	70.0	75.0	80.0	85.0	90.0	95.0	100.0
45	0.1	0.2	0.3	0.5	1.0	2.0	3.0	5.0	10.0	15.0	20.0	25.0	30.0	35.0	40.0	45.0	50.0	55.0	60.0	65.0	70.0	75.0	80.0	85.0	90.0	95.0	100.0
46	0.1	0.2	0.3	0.5	1.0	2.0	3.0	5.0	10.0	15.0	20.0	25.0	30.0	35.0	40.0	45.0	50.0	55.0	60.0	65.0	70.0	75.0	80.0	85.0	90.0	95.0	100.0
47	0.1	0.2	0.3	0.5	1.0	2.0	3.0	5.0	10.0	15.0	20.0	25.0	30.0	35.0	40.0	45.0	50.0	55.0	60.0	65.0	70.0	75.0	80.0	85.0	90.0	95.0	100.0
37	0.1	0.2	0.3	0.5	1.0	2.0	3.0	5.0	10.0	15.0	20.0	25.0	30.0	35.0	40.0	45.0	50.0	55.0	60.0	65.0	70.0	75.0	80.0	85.0	90.0	95.0	100.0
38	0.1	0.2	0.3	0.5	1.0	2.0	3.0	5.0	10.0	15.0	20.0	25.0	30.0	35.0	40.0	45.0	50.0	55.0	60.0	65.0	70.0	75.0	80.0	85.0	90.0	95.0	100.0
39	0.1	0.2	0.3	0.5	1.0	2.0	3.0	5.0	10.0	15.0	20.0	25.0	30.0	35.0	40.0	45.0	50.0	55.0	60.0	65.0	70.0	75.0	80.0	85.0	90.0	95.0	100.0

TABLE 3. *Destruction of pentosan by cooking*

Sample No.	TREATMENT					Pentosan in sample	Pentosan found after cook gms.	Pentosan decomposed gms.	Pentosan decomposed percentages
	Conc. NaOH percentage	Vol. NaOH cc.	Pressure of cook in lbs.	Time of cook min.					
1	1.0	50	35	30	0.3502	0.3223	0.0279	7.95	
2	2.0	50	35	30	0.3500	0.3147	0.0353	10.10	
3	3.0	50	35	30	0.3511	0.2918	0.0593	16.90	

added (250 cc. for the fibrous material, 750 cc. for the pith). The beaker was brought to boiling on an electric hot plate at a constant rate and held at the boiling temperature for thirty minutes, the volume being kept constant by the addition of water. The hot alkaline extract was filtered from the tissue by suction through a cloth filter, the tissue was washed until the washings coming through were colorless. An aliquot of the combined liquor and washings was taken for pentosan determination by furfural distillation and another aliquot was taken for the determination of total organic matter extracted from the stalk, the latter being made by evaporating the solution at 110 degrees and burning out the organic matter.

The tissue after washing was transferred with 400 cc. of water and stirred until uniformly distributed. The tissue was then subjected to the action of chlorine gas for the indicated time, the rate of chlorination being controlled by a flowmeter delivering the gas stream. After chlorination, the liquor was filtered off and the tissue washed until the washings were free from chlorine. Aliquots of the combined liquor and washings were run for pentosan and total organic matter removed from the stalk.

The tissue was finally treated with 500 cc. of sodium sulfite solution of the indicated strength, the suspension brought to boiling and boiled for five minutes. The hot sodium sulfite extract was filtered off and the residue washed until the washings coming through were colorless. Aliquots of the combined liquor and washings were run for pentosan and total organic matter removed.

The tissue resulting from these three treatments was bleached with permanganate solution followed by sulfurous acid solution, dried in a vacuum oven at 60°, and analyzed for ash, pentosan, lignin and α -cellulose content.

DESTRUCTION OF PENTOSAN BY COOKING

The total pentosan accounted for in no case checked the amount, approximately 26 per cent, in the original tissue. Experiments in which pentosan, extracted from cornstalks with 5 per cent sodium hydroxide solution, was treated with caustic solution indicated a corresponding destruction, the destruction being greater the higher the concentration of caustic. The data are shown in table 3. During the progress of this investigation, an article appeared by Aronovsky and Gortner (6) which shows similar results when wood is treated with water alone. This destruction is possibly due to the dehydration of the pentosan material with the production of furfural.

DISCUSSION OF RESULTS

The quantity of organic matter removed by each of the three treatments indicated that the initial caustic treatment was the only variable which removed appreciable quantities of material, although the quantity of organic matter removed increased slightly with increased time of chlorination as well as increased concentration of sodium sulfite. This result is in harmony with the work of Ritter (7) which indicates that a short period of chlorination is sufficient to chlorinate all the exposed lignin. In the treatment of the pith as well as in the treatment of the fibers an increase in caustic concentration caused a decrease in the yield of pulp, α -cellulose content, lignin content, and pentosan content of the pulp and an increase in the organic matter removed.

In the case of the fibers, the 2.0 per cent caustic treatment produced a good white pulp while the 1.0 per cent and 0.5 per cent caustic treatment produced a dark colored insufficiently treated pulp. On testing intermediate concentrations, it was found that 1.2 per cent caustic failed to give an entirely satisfactory pulp while 1.3 per cent caustic gave a satisfactory pulp. This calculated to be 12.5 per cent sodium hydroxide on the weight of the dry fibers. The actual dilution of the caustic solution has little effect for a pulp may be obtained from as dilute a solution as 0.5 per cent caustic concentration when the ration of dry fiber tissue to caustic solution is 1 to 30.

In the case of the pith material, the 2.0, 1.0, and 0.5 per cent caustic treatments all gave satisfactory pulps. On testing lower concentrations, it was found that a satisfactory pulp was obtained with 0.3 per cent but not with 0.2 per cent caustic solution when 30 cc. caustic solution were used per gram of material. This calculated to be 7.5 per cent sodium hydroxide on the dry material.

The lignin left in the pulp tends to approach a minimum value with increased caustic concentration, however, it is not completely removed.

When a good pulp is produced, the α -cellulose content is from 35 to 40 per cent of the total stalk. This value tends to approach a constant minimum value as the concentration of reagents increases.

SUMMARY

The cornstalk can be mechanically separated into a pith tissue and a fibrous tissue, yielding 63 per cent fibrous constituents and 26 per cent pith based on the weight of the original stalk.

In the de Vains process, when applied to the pulping of cornstalks, the initial caustic treatment is the only variable which has any appreciable effect on the chemical composition of the pulp.

The concentration of the caustic solution used makes very little difference so long as there is caustic enough present for pulping. The fibrous tissue of the stalk requires 12.5 per cent sodium hydroxide, based on the dry weight of the fibers, for pulping while the pith tissue requires only 7.5 per cent sodium hydroxide based on the dry weight of the pith.

Isolated pentosan material is destroyed by pulping processes, the destruction being greater the greater the caustic concentration of the cook.

The percentage of α -cellulose as well as lignin in the pulp tends to approach a constant minimum value. However, the removal of lignin is in no case complete.

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THE USE OF AMMONIUM HYDROXIDE AS A PULPING AGENT FOR THE GRASSES

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In the process of converting plant tissues into cellulosic pulp by chemical means, approximately fifty per cent of the original tissue is recovered. There has been no method as yet suggested which would permit the recovery of the other fifty per cent of the organic material at an economical figure. On an estimated annual production of three million tons of paper pulp from the various chemical processes, this destruction would amount to about one and one-half million tons of organic matter per year for the United States alone.

The following report presents preliminary evidence to show that ammonium hydroxide could be substituted for sodium hydroxide in the alkali-pulping processes for the grasses. The ammonia can be recovered by distillation leaving the organic materials of the black liquor as a residue for further elaboration. This process would offer commercial advantages in proportion to the uses found for the organic materials recovered, consisting mostly of lignin and pentosans.

MATERIALS AND LABORATORY PROCEDURE

The plant materials used in these studies consisted of oat straw and the fibrous tissue of the cornstalk. The method of separation of the cornstalk is described in a previous publication.¹ The fibrous tissue was used since it is so much more difficult to pulp than the pith tissue that any pulping process which would pulp the fiber would also pulp the pith.

The cornstalk tissue was used in the air dry condition in the same state as it came from the separator. The material had a moisture content of 5.63 per cent on an oven dry basis of 105°C. The dry tissue ran 1.55 per cent ash.

The oat straw tissue was used in the air dry condition in the same state as it came from the bales. The material had a moisture content of 6.80 per cent on an oven dry basis of 105°C. The dry tissue ran 3.77 per cent ash.

A special laboratory type of digester was used for this investigation. A schematic diagram is shown in figure 1. The four heat units made it possible to maintain any desired temperature since they could be connected in series or in parallel, and also cut out entirely when necessary. The digester was lead lined in order that it might be used for sulfite cooks. When sulfite cooks were made, the iron head was replaced by a silver plated head. The lead lining made it possible to get a tight joint at the top.

The tissues were subjected to a modification of the de Vains process² in that the base used was ammonium hydroxide instead of sodium hydroxide. One hundred gram samples were used. The material was placed

¹ Peterson, Fang, and Hixon, Iowa State College Jour. Sci. 7: 17-24, 1932.

² See discussion and literature cited p. 23 of this journal.

in the digester and the smallest volume of aqueous ammonia of the indicated concentration sufficient to cover the sample (1000 cc.) was added. The pressure in the digester was slowly brought to 90 to 110 pounds and held at this pressure from 8 to 15 hours by use of the electric heat units. After the digester had cooled the pressure was decreased to atmospheric pressure by opening the blow-off valve which removed practically all the liquor from the digester. The digester was opened and the pulp thoroughly washed with water.

The washed tissue was transferred to a laboratory sized paper beater and beaten until a good pulp was obtained, then treated with chlorine gas for 20 minutes or until the tissue lost the brown color and became a faint yellow. After chlorination the liquor was filtered off, the tissue washed until free from chlorine, then sufficient two per cent sodium sulfite was added to form a heavy suspension and the mixture boiled for five minutes. The hot sodium sulfite extract was filtered off and the residue washed on the filter until the washings came through colorless.

The tissue resulting from these treatments was bleached with permanganate solution followed by sulfurous acid solution. A portion was dried in a vacuum oven at 60°C. and analyzed for ash, pentosan, lignin, and cellulose content. The results of these analyses are shown in table 1. Another portion was made into paper. This paper was of such a quality as to compare with the de Vains process or soda process papers.

OBSERVATIONS ON WASTE LIQUORS

The ammonia from the waste liquors volatilizes to a large extent in "blowing off" from the digester. Aeration during boiling reduces the ammonia concentration in the waste liquor to a few tenths of a per cent. As the ammonia volatilizes, lignin separates from the liquors but filtration is made difficult due to the peptizing action of the soluble pentosans. Removal of all the water by evaporation leaves a black sludge containing pentosans and lignin. The analysis of these mixtures for total organic matter and for pentosans is shown in table 1. The difference between these figures would be a rough indication of lignin content.

TABLE 1. *The partition of the constituents of plant tissues by ammonium hydroxide as compared with sodium hydroxide**

Kind of tissue and process	Yield of pulp	Analysis of pulp			Analysis of liquor	
		Pentosan	Lignin	α -Cellulose	Total organic:	Pentosans
Cornstalk + NH_3	59	17.4	2.3	39	30.3	7.1
" + NaOH	52	14.4	2.2	38.2	33.6	8.4
Oat straw + NH_3	57	19.0	2.5	39	32.2	8.3
" " + NaOH	52	16.2	2.1	36	32.3	9.1

* One hundred grams of tissue cooked in one liter of 28 per cent ammonia at 100 lbs. pressure for 15 hours. Results are reported as percentage of original tissue.

The pentosans can be removed from the lignin by dilute acid hydrolysis. Light tan colored preparations of lignin can be obtained by this method. Further studies of this material will be reported in the near future.

The pentosans can be recovered from the ammoniacal solutions by precipitation with alcohol according to standard procedures for prepara-

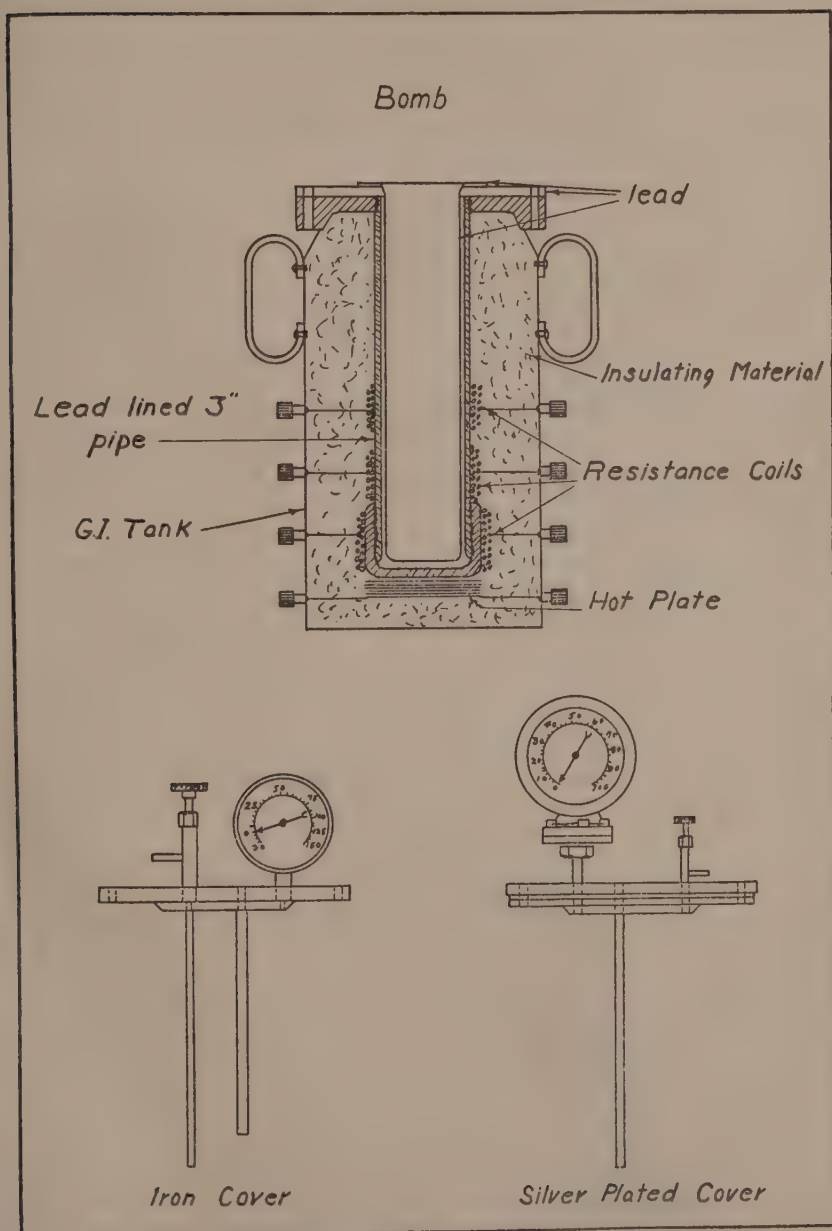


Fig. 1. Laboratory digester.

tion of pentosans from sodium hydroxide extracts. After removal of the pentosans, lignin can be separated without the filtration difficulties just mentioned.

Since the isolated pentosan materials can be easily fermented by the thermophilic organisms, it was expected that the lignin could be separated from the carbohydrate materials in the waste liquor by subjecting the evaporated waste liquors to thermophilic digestion. Little or no growth could be obtained in such suspension media.

SUMMARY

The analyses reported in table 1 indicate that a paper pulp of similar composition can be obtained from cornstalks or oat straw independently of whether ammonium hydroxide or sodium hydroxide is used as a pulping agent in the de Vains process. The actual yield of pulp is slightly higher from the ammonia cook because of larger quantities of pentosans left in the pulp. Papers prepared from the pulps showed very similar qualities. The paper from the ammonia process seemed stronger and less hydrated probably due to the milder alkali and lower temperatures (about 115°C.) employed.

The organic material in the liquors was about 25 per cent pentosan and 75 per cent lignin. These constituents may be separated by the usual procedures. The ammonia can be recovered by boiling and aeration of the waste liquors. About 0.5 per cent ammonia remains in salt form after such recovery and it is necessary to add small quantities of strong base, for example Ca(OH)_2 , to effect complete recovery.

THE HYDROLYSIS OF PENTOSANS FROM CORN COBS

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La Forge¹ has investigated the production of furfural from corn cobs by steam distillation. The furfural is supposed to be formed from the pentosans which are present in the cobs to the extent of about 30 per cent of their mass. He found that a ratio of cobs to water not greater than 1:4 a temperature of 180° and a reaction period of two hours were optimum conditions. If steam carrying furfural was allowed to escape during the two hour period the yield of furfural was increased. Under any conditions imposed by La Forge a large fraction of the pentosans were converted into products other than furfural or much of the furfural which was formed was subsequently decomposed, since he seldom secured as much as one-third of the theoretical yield. Since the effect of the other components of the cob were unknown, it was decided to remove the pentosans from the major part of the other components and conduct hydrolyses of less impure pentosans.

PREPARATION OF PENTOSANS

The pentosans were extracted by adding fine corn cob meal to a 7 per cent solution of sodium hydroxide until the mixture was as viscous as could be stirred conveniently with a motor stirrer. The mixture was stirred for one hour, allowed to stand over night, stirred for another hour and then filtered under suction. The filtration was made through a towel supported on a Buchner funnel. The residue was washed with water and the washings were added to the filtrate. The filtrate was stirred while two volumes of 95 per cent ethanol were added, and after complete mixing the sodium hydroxide was partly neutralized by means of hydrochloric acid. After two or three hours the precipitated pentosans were collected on a filter, washed with alcohol and ether, dried in the air for three or four hours, and stored in a tightly stoppered bottle. Their composition was:

Moisture (loss at 105°)	17.03 per cent
Pentosans (furfural distillation)	71.05 " "
Ash	6.86 " "
Lignins, etc. (by difference)	5.06 " "
	<hr/> 100.00

APPARATUS

The bomb in which the hydrolyses were made is shown in figure 1. It consisted of a special steel cylinder three and one-half feet long with a bore three inches in diameter and a wall thickness of one inch. It was permanently closed at one end and the other end was closed by a steel head, *A*, and a lead gasket held in place by stud bolts. The bomb was mounted by means of an axle, *B*, on which it could be rocked by a motor,

¹ Ind. Eng. Chem. 15, 499 (1923)

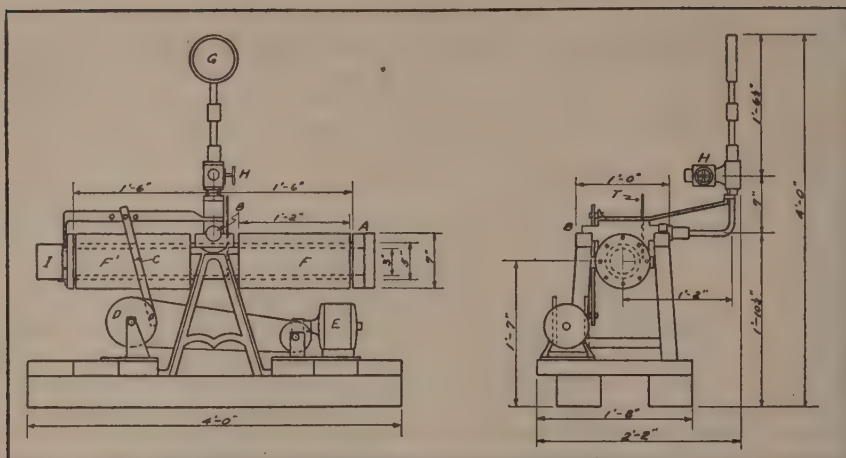


Fig. 1. Rocking perssure bomb. Scale: 1" = 3'-0"

E, eccentric, *D*, and shaft *C*. The variable weight, *I*, aided in balancing the apparatus. The hollow axle communicated with the interior of the bomb and to it was connected a pressure gauge, *G*, and a connecting tube fitted with a valve, *H*. The bomb was heated by the electrical furnaces *F*, *F'*. The temperatures were read by means of a thermometer, *T*, set in a well drilled in the steel collar which connected the bomb to the shaft. The relation between the thermometer reading and the temperature in the bomb was determined by filling the bomb with nujol, fitting a thermocouple so that its junction was in the middle of the bomb, and reading the thermocouple and the thermometer in the well simultaneously as the bomb was heated through a range which included the temperatures of these experiments.

EXPERIMENTAL

Fifteen grams of air dried impure pentosans, (10.66 grams by furfural distillation) and 2,500 cc. of water were put in the bomb, sealed, and heated to the boiling point before the valve, *H*, was closed. This permitted the greater part of the oxygen to be displaced. The sealed bomb was rocked continuously during a 70-120 minute period of rising temperature, a five hour period of constant temperature and until the bomb was cooled by radiation. When the bomb was cool the contents were poured out and the bomb was washed with water. The liquor and washings were brought to 3,000 cc. by adding distilled water, and aliquot parts of this 3,000 cc. were analyzed.

ANALYSIS

Furfural was collected from 1,000 cc. by distillation under reduced pressure until aniline reagent gave no pink color with the last distillate. The furfural was precipitated from 12 per cent hydrochloric acid by phloroglucinol, weighed, and the calculations made by Kröber's formulas.²

² Schorger, Chemistry of Cellulose and Wood. McGraw-Hill Book Co., New York (1926) p. 536.

ALCOHOL INSOLUBLE MATERIAL

After the furfural had been removed the concentration was continued under reduced pressure until the volume was about 300 cc. After cooling, a representative portion was taken and two volumes of 95 per cent ethyl alcohol were added. After two or three hours, the precipitate was collected under suction on a small tared Buchner funnel. The precipitate was first washed with alcohol and then with ether and dried in an oven at 105°C.

ALCOHOL SOLUBLE MATERIAL

The alcohol from the filtrate was recovered by distilling under reduced pressure. To a 20 cc. representative portion of the remaining liquor, concentrated hydrochloric acid was added until the concentration of hydrochloric acid was 12 per cent. The volume was then made up to 100 cc. with 12 per cent hydrochloric acid. This was distilled, at the rate of 30 cc. in 10 minutes, until the distillate gave no pink color with aniline reagent. The volume in the distillation flask was kept constant by addition of 12 per cent hydrochloric acid. The amount of furfural in the distillate was determined in same way as described above.

Reducing substances not removed when furfural was removed. A 50 cc. representative portion of the concentrate, after the furfural was removed, was clarified with a saturated lead acetate solution, and delead with dry sodium carbonate. Before adding the sodium carbonate a drop of phenolphthalein was added so that the appearance of a slight pink color would indicate when enough sodium carbonate had been added. The reducing material calculated as glucose was determined by the Modified Fehling Solution method of Quisumbing and Thomas.³

Volatile acids. Volatile acids were determined by the method used by Aronovsky and Gortner.⁴

RESULTS

A series of seven runs was made. The temperature of the runs varied from 142° to 208°C. Table 1 shows the results of these seven runs.

DISCUSSION OF RESULTS

The liquors from the various runs differed in color. The liquors from 142° and 150°C. runs were yellow, those from the 160° and 173°C. runs were dark brown, those from the runs above 173°C. were black and had an odor like that of charred organic matter.

The yield of furfural reached its maximum of 5.03 per cent, of the theoretical yield, in the sample heated to 173° for 5 hours. In the runs above 173°C. the yield decreased gradually.

The yield of volatile acids was small and reached a maximum at about the same temperature as the furfural.

The alcohol insoluble material was greatest in the low temperature runs in which the yield of furfural was smallest. In the low temperature runs the alcohol insoluble material looked very much like the original material. In the runs at higher temperatures the amount of alcohol insoluble material was very small and the material was black.

³ J. Am. Chem. Soc. 43, 1503 (1921).

⁴ Ind. Eng. Chem. 22, 264 (1930).

TABLE 1. *Analysis of liquors from five hour runs at varying temperatures*

Maximum temperature Degrees centigrade	Pressure Pounds per sq. inch	Furfural Grams	Pentosans accounting for furfural Grams	Volatile acids calculated as acetic acid Grams	Alcohol insoluble material Grams	Reducing material other than furfural calculated as glucose Grams	Alcohol soluble material	
							Furfural Grams	Furfural producing material calculated as pentosans Grams
142	40	0.009	0.000	0.225	9.005	0.000	0.644	1.115
150	65-70	0.072	0.124	0.354	6.080	0.000	1.850	3.164
160	80-85	0.306	0.554	0.773	1.660	1.215	1.737	2.970
173	115-125	0.390	0.567	0.906	0.860	0.757	trace	trace
184	160	0.297	0.507	0.809	0.684	0.651	0.000	0.000
196	185	0.189	0.326	0.773	0.804	0.380	0.000	0.000
208	280	0.183	0.213	0.773	0.632	0.082	0.000	0.000

The alcohol soluble portion was richest in furfural producing material in the low temperature runs in which the yield of furfural was smallest. In the high temperature runs there was no furfural producing material at all. This would indicate that in the runs at lower temperatures, the treatment had not been sufficient to convert the pentosans to pentoses or furfural.

Reducing material other than furfural was greatest in the 5 hour run at 160° and decreased with increase in temperature.

A considerable portion of the pentosans was unaccounted for in the various experiments. This might be due to the formation of furfural and its subsequent decomposition or to the decomposition of the pentosans without the formation of furfural.

SUMMARY

Pentosans, extracted from corn cobs, were treated with water in a gas tight bomb. The pressures developed were those corresponding to the vapor tension of the materials present at the temperature of the various experiments. The products determined were furfural, volatile acids, alcohol insoluble material, furfural producing material in alcohol soluble portion, and reducing material other than furfural.

The yield of furfural reaches its maximum in 5 hours at 173°.

The amount of alcohol insoluble material decreases with increase in temperature. When pentosans were treated at temperatures below 173° the alcohol extract contained considerable furfural producing material, while those samples treated at higher temperatures yielded little or no furfural producing material. In no case do the products determined account for all of the pentosans. In the experiments at the higher temperatures the products identified account for only a small portion, less than 5 per cent, of the pentosans used.

THE LIFE HISTORY AND HABITS OF *IXODES SCULPTUS* NEUMANN (IXODIDAE)

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The sculptured tick, *Ixodes sculptus* Neumann (1904), is one of the few species of the genus that have received very little mention in the literature. So little attention has been given to this species that the male is recorded as unknown, while the female is known only from a few specimens that have been available for authoritative consideration.

Although this work is not primarily concerned with the systematic phase, the abundance of available material has yielded some information that may be of interest to the systematist.

The life history and habits of *Ixodes sculptus* have been investigated primarily as a contribution to knowledge concerning the Ixodidae. The association of this tick in nature, however, places it as a possible vector of tularaemia. Hence, this work was planned that it might be of service in investigating this tick as a vector of disease.

The time of the year to which this study was necessarily restricted has made it impossible to carry on extensive investigation in the tick's natural habitat. Consequently, the life history and habits have been worked out almost wholly under laboratory conditions. Some observations made in the field, however, have been used to verify and supplement conclusions drawn from laboratory data.

LITERATURE

Ixodes sculptus was described by L. G. Neumann in 1904. Hunter and Hooker (1907) mention only the scarcity of specimens. A paper by F. C. Bishopp (1911) designates the distinct systematic characters and the extent of the known distribution at that time. This brief review of the literature is apparently complete.

GEOGRAPHICAL DISTRIBUTION AND HOST RELATION

Ixodes sculptus is restricted to North America. It is more widely distributed on this continent, however, than the scarcity of specimens would lead one to suspect. The type locality is the Santa Cruz Mountains of California. Specimens are recorded from the prairie dog and the rock squirrel (*Citellus variegatus* Couchi) in Texas, the ground squirrel (*Citellus columbianus*) in Montana and the striped-ground squirrel in Wyoming. A specimen is recorded from South Dakota, but no host is mentioned. A decapitated specimen from the bob cat in Oklahoma is recorded as *Ixodes sculptus*, but its determination is apparently incorrect. *Ixodes sculptus*

¹ The writer has been greatly aided during the progress of this work by constructive criticism and suggestions kindly given by Dr. E. E. Becker.

In the systematic study, the author was fortunate in having specimens submitted by Professor C. E. Sanborn of Oklahoma A. and M. College. The determination of the species was courteously made by Mr. F. C. Bishopp, of the United States Bureau of Entomology.

has been collected recently from the pocket gopher at Stillwater, Oklahoma, by Professor C. E. Sanborn. The specimens used in this study were taken from the thirteen-striped ground squirrel (*Citellus tridecemlineatus tridecemlineatus*) in the vicinity of Ames, Iowa.

DESCRIPTION

Male (figures 2 and 3): Length (excluding capitulum), 1.87 to 2.45 mm.; width, 1.44 to 1.58 mm. Body oval, greatest width slightly anterior. Marginal fold white, prominent, set with stout setae; marginal groove deep. Scutum coarsely punctate, setae scarce. Cervical grooves faint, strongly diverging. No lateral grooves. Pseudo-scutum faintly evident. Two small sclerotized areas adjacent to internal spine of coxa I. Pregenital plate anteriorly rounded. Median plate as broad as long. Anal groove slightly diverging posteriorly. Adanal plates with sides parallel, diverging at anterior extremity. Spiracle subcircular, measuring from .21x.24 to .24x.27 mm. Capitulum small. Basis capitulum narrowing posteriorly forming small cornua. Palpal segments 2 and 3 equal. Hypostome bluntly rounded, teeth fused transversely. Coxa I with a strong internal spine projecting over Coxa II. A blunt external spur on all coxa. Anterior legs noticeably stouter than others. Tarsi tapering abruptly.

Female: Body size (excluding capitulum) ranging from 1.44X2.23 to 1.69X2.47 mm. Sclerotized parts dark amber in color. Unsclerotized area whitish marked by slate colored diverticula of intestine. Lateral carinae curving medially. Porose areas rounded, almost covering caudal half of basis capitulum. Internal spine of coxa I long, extending over coxa II. Hypostome set ventrally with two rows of teeth. Measurements from 36 individuals: Scutum, 1.20X1.05 to 1.37X1.17 mm.; capitulum (ventral ridge to tip of hypostome), .69 to .81 mm.; (dorsal ridge to tip of hypostome), .55 to .69 mm.; basis capitulum, .409 to .472 mm.; spiracle, .254X.197 to .296X.225 mm.; cornua centers, .289 to .351 mm.; sculpulae interval, .473 to .486 mm. Engorged female somewhat rounded in form, slate blue in color. Length including capitulum, 6.3 to 8.6 mm.; width 4.4 to 5.4 mm.

Nymph: Body oval, narrower in front; translucent, marked by diverticula of intestine. Sclerotized parts amber in color. Marginal groove shallow. Cornua prominent. Anterior legs very stout in comparison to others. Palpal segment I with two prominent spines. Unengorged (excluding capitulum), 1.203X.705 to 1.332X.788 mm. Engorged, 2.23 to 1.50 mm.

Larva: Body oval, translucent, marked light brown by diverticula of intestine. Sclerotized parts amber in color. No marginal groove. Cornua prominent. Palpal segment I with two prominent spines. Unengorged (excluding capitulum), .60X.48 to .64X.49 mm. Engorged, .87X1.39 mm.

Egg: Ellipsoidal, amber color. Average size of 30 eggs, .428X.614 mm.

TECHNIQUE

The devices used in carrying out this study have given such excellent results that descriptions and methods of using them are given for the benefit of future workers.

For infesting the ground squirrel, the animal was confined in a mailing tube about three and one-fourth inches in diameter and eight inches long. Several small holes were made in the lid of the tube for ventilation and for admitting the unengorged ticks. Three hours after the ticks were given access to the host, the squirrel was removed from the mailing tube and placed in a one-fourth inch mesh hardware cloth cage placed over a pan of water. The engorged ticks after detaching from the host were recovered from the pan of water. Infestations ranging from 40 to 80 per cent were obtained in this way.

The development of engorged larvae and nymphs was completed in a device (fig. 1) constructed as follows: A small vial about three-fourths inch in diameter and three inches long was filled about one-fourth full of sand saturated with water. A one-fourth inch glass tube was inserted through a cork so that it extended almost to the sand when the cork was fitted into the vial. Cellu-cotton plugs were used to stopper each end of the glass tube. The engorged larvae and nymphs were placed in the tube between the cellu-cotton plugs for further development. Variations from the principle of this device were used, but they proved to be less efficient.

Oviposition was carried out in small Stender dishes containing a thin layer of fine moist sand. The engorged females were placed on the moist sand and the lid of the dish put in place for moisture retention. Only one small cluster of eggs handled in this way was lost from the attack of molds.

LIFE HISTORY

No previous work on the biology of this tick has been published. With the exception of the parasitic interval, the complete life history was carried out under a constant temperature of 24.5 C. For comparison, the development of the larva and nymph was carried out under a constant temperature of 22.5 C. All stages were experimented with under freezing and unrecorded room temperatures. All engorgements were made on the thirteen-striped ground squirrel.

OVIPOSITION

The preoviposition period of fourteen females ranged from five to nine days. At 24.5 C., one specimen began ovipositing in five days and four others began on the sixth day. Under variable room temperature, six began ovipositing in six days, two in seven days and one in nine days. Two individuals under a constant temperature of 34.7 C. failed to oviposit in fourteen days. Oviposition began, however, eight days after they were changed to 24.5 C. The eggs were abnormal and failed to develop. An engorged female subjected to -3 C. for fourteen days began ovipositing the fourth day after removal to 24.5 C.

The number of eggs deposited by individuals ranged from 644 to 1,006 with an average of 828 from five specimens. The number of eggs

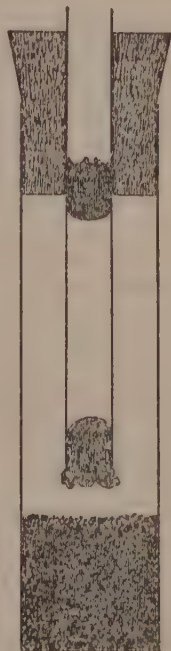


Fig. 1. Apparatus for rearing non-parasitic stages of immature ticks. (Original)

deposited showed a positive correlation to the weight and size of the engorged female (as shown in Table 1). Beginning with the day oviposition began, the average number of eggs deposited by five individuals on subsequent days were as follows: 19, 61, 81, 79, 74, 77, 68, 48, 43, 50, 36, 35, 30, 24, 21, 16, 16, 11, 10, 8, 8, 7, 4, 4, 2, 1, 1, 1 and 1. The oviposition period ranged from twenty-six to twenty-nine days.

TABLE 1. *Relationship of the engorgement period and eggs deposited to size and weight*

Engorgement period	Length in mm.	Width in mm.	Weight in grams	Number of eggs
6 days	7.2	4.8	.0556	644
14 days	7.6	4.8	.0716	768
12 days	7.9	5.2	.0831	789
10 days	8.0	5.2	.0967	935
7 days	8.2	5.1	.0955	1006
8 days	8.0	5.2	.0950	none
8 days	8.6	5.4	.1001	none

The incubation period, under varying temperatures, ranged from twenty-seven to forty-three days. Eggs deposited on subsequent days by the same individual and maintained at 24.5° C. showed distinct white spots on the twenty-first day and began hatching on the thirty-fourth day after the date of oviposition. There was a four day variation in the length of the incubation period of eggs from different individuals. The development of eggs from the majority of individuals, however, was constant. Eggs subjected to -3° C. for fourteen days developed normally when removed to effective temperatures. Also, eggs from an engorged female that had been subjected to -3° C. for fourteen days were normal and began hatching in thirty-four days after removal to 24.5° C.

LARVA

The longevity record for the unengorged larva was 166 days at 24.5° C. Of ten larvae that hatched November 15, 1931, four were still living on May 1, 1932. Under normal conditions, engorged larvae dropped from the host in from three to nine days after attachment. Beginning with the second day after infestation, the numbers of engorged larvae dropping from the host on subsequent days were as follows: 9, 40, 56, 56, 64, 72, 74, 28, and 3. The engorgement of 149 larvae on one squirrel from the same infestation, increased the range of the engorgement period four days; beginning with the third day after infestation, the following numbers detached on subsequent days: 7, 33, 38, 32, 22, 37, 12, 16, 9, 6, and 2. Engorgement was retarded when a squirrel went into hibernation the sixth day after infestation.

Engorged larvae held at both 24.5° C. and 22.5° C. became quiescent in from three to seven days after dropping from the host. Quiescence was evident in over 50 per cent on the fourth day. At room temperature, quiescence took place in four to nine days with 50 per cent becoming inactive on the fifth day. Active engorged larvae subjected to -3° C. for fourteen days became quiescent in three to six days after removal to 24.5° C.

Quiescent larvae at 24.5° C. began clearing at the base of the capitulum on the second day and molted in from nine to twelve days. Those

subjected to 22.5° C. molted on the twelfth and thirteenth day. A one day possible error may account for the variation in the period required for development. Molting occurred in from thirteen to seventeen days at room temperature. A temperature of -3° C. only retarded development in quiescent larvae.

TABLE 2. *Larva and nymph development of Ixodes sculptus under constant temperature and optimum humidity*

Stage	Temperature	Number of specimens quiescent Days after dropping							Number of specimens molting Days after quiescence								
		3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
Larvae	24.5 C.	8	45	21	17	6	1	13	61	24	5	1					
Larvae	22.5 C.	4	44	17	7	1	1				22	29	3	1			
Nymph ♂	24.5 C.	1	7	11	3	1				10	8	1					
Nymph ♀	24.5 C.	1	9	7	8	4				1	12	17	1				
Nymph ♂	22.5 C.			3	1	3					1	6					
Nymph ♀	22.5 C.			2	2	2								2	3	1	

NYPH (TABLE 2)

The longevity record of the nymph, in the unengorged condition, was 118 days. Mold was a disturbing factor in this experiment; therefore, it is probable that this form is capable of enduring much longer. Engorgement was completed under normal room temperature in from four to nine days after infestation. Starting with the third day, the number of engorged nymphs dropping on subsequent days were as follows: 1, 4, 13, 14, 15, 8, 10, 5 and 1. Cool temperatures and poor condition of the host prolonged the period required for engorgement. Hibernation of the squirrel retarded the engorgement of attached nymphs.

Quiescence was evident in engorged nymphs in from three to seven days at 24.5° C., at 22.5° C. in from five to seven days. Those subjected to room temperature variations remained active for from eight to sixty-four days. The extended period was probably due to low temperature fluctuations. No complete record of the temperature was taken, but records from noticeable variations showed a range from 18 to 30° C. There was no difference in the length of the active period of engorged male and female nymphs under optimum conditions.

Quiescent nymphs subjected to the same conditions showed a distinct difference in the time required for molting of the different sexes (as shown in table 2). At 24.5° C., the males molted on the eleventh and twelfth day and the females molted on the twelfth and thirteenth day after quiescence. At 22.5° C., the males molted in fourteen days and the females molted in from fifteen to sixteen days. At room temperature, quiescent nymphs molted in from sixteen to nineteen days.

In a few instances where both temperature and humidity were not optimum, the period from detachment to molting ranged from seventeen to twenty-nine days for females and twenty-one to fifty-six days for the males. During development, the males are capable of withstanding more adverse humidity conditions than the females. Out of twelve engorged nymphs held at fluctuating humidity, five molted, which were all males; whereas, out of thirty-nine held at optimum humidity two died, and thirty-

seven molted, of which seventeen were males and twenty were females. The regenerations of lost appendages increased the period required for development from one to two days.

ADULT

Specimens collected in the field were used in the longevity test of the adult stage; therefore the duration of life is probably much longer than the data indicate. At temperatures suitable for activity, an unengorged female lived 131 days and several males were still alive after 182 days. The time required for engorgement of the female ranged from six to fourteen days. The condition of the host is a factor influencing the time required for engorgement and the size of the engorged tick, as shown in table 1. The specimens represented in this table attached the same day on a single host. The individual engorging in six days probably detached before repletion. The two specimens engorging in twelve and fourteen days were below the common range in size. This view was further substantiated by the fact that the seventh engorged adult that detached from a field collected squirrel, measured only 6.25X4.42 mm. This specimen detached nine days after the squirrel was collected. A single female attaching to this squirrel later required fourteen days to engorge.

HABITS

All stages of *Ixodes sculpus* are adapted for life in the burrow, and except for the unengorged larvae, they have been collected in the litter of the burrow of the thirteen-striped ground squirrel.

The parasitic forms of this species infest the host from the burrow. Although unengorged larvae have not been collected in the burrow, the gravid females and eggs collected in the burrow account for its existence there. The unengorged forms apparently remain inactive in the burrow until disturbed by the presence of the host. They crawl from the litter or fall from the sides or roof of the burrow onto the host and attach along the back and about the head and neck. All engorged forms leave the host at night while the host is inactive and burrow down into the litter for molting or oviposition. The male is not parasitic; it remains in the litter of the burrow and copulates either before or after the engorgement of the female. Whether the engorged female is fertilized or not, it burrows into the surface of the soil below the litter of the nest and deposits the eggs after the usual preoviposition period.

LIFE CYCLE

Oviposition usually begins on the sixth day after engorgement and as many as 1,006 eggs may be deposited. Twenty-eight days are required for oviposition. At 24.5° C., the eggs hatch thirty-four days after the date of deposition. The larva may live in the unengorged condition at least 166 days and engorge as soon as three days after gaining the host. At 24.5° C., it becomes quiescent in four days and molts ten days later.

The nymph may live at least 118 days in the unengorged state and engorge within four days after attachment. At 24.5° C., the nymph becomes quiescent in five days; at this temperature the male molts in eleven and one-half days, and the female in twelve and one-half days. The unengorged female is capable of living at least 131 days, and usually en-

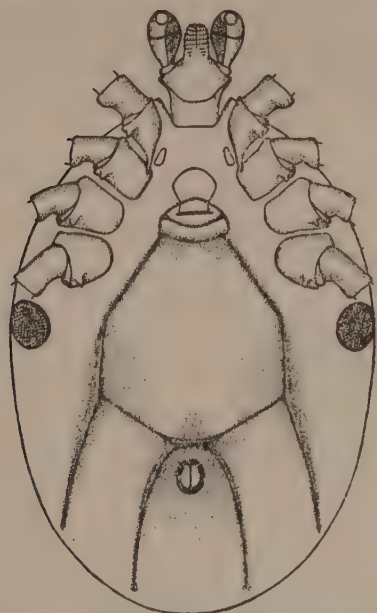


Fig. 2. *Ixodes sculptus*. Ventral view of male, greatly enlarged. (Original)

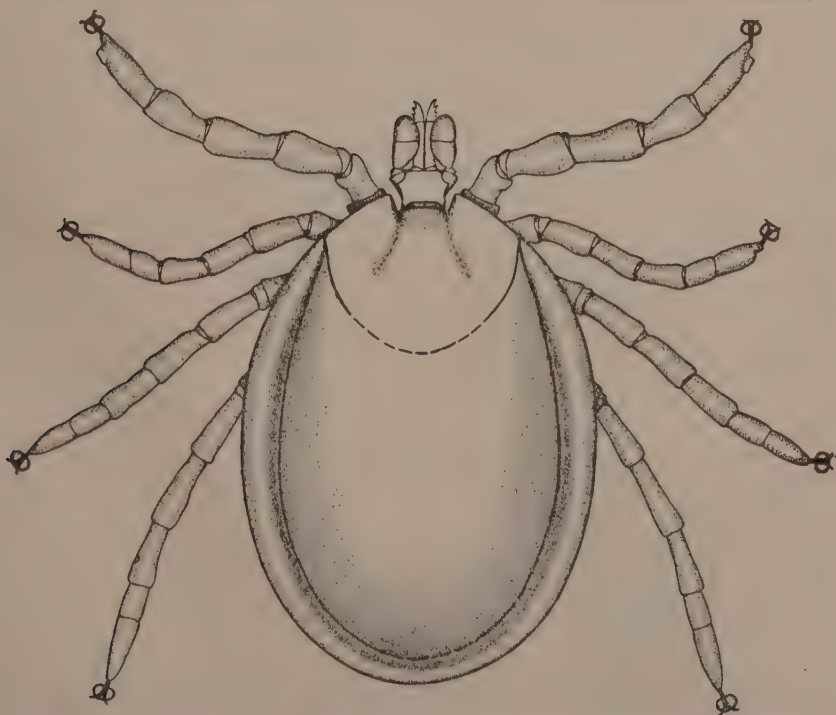


Fig. 3. *Ixodes sculptus*. Dorsal view of male, greatly enlarged. (Original)

gorges in about eight days after attachment. The male is capable of living 182 days at temperatures suitable for activity.

All stages of this tick may be found at any time during the year. It has been shown from field collecting that all forms are present from early fall to late spring.

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STRUCTURE AND CONTENT OF THE POLLEN OF SOME LEGUMINOSAE

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This paper deals with the structure and content of the pollen of 41 species distributed among 26 genera of the Leguminosae. All these species are visited by bees. Most of them are sources of both pollen and nectar.

The morphological features considered are size and shape of pollen; thickness of wall; number and character of the furrows; number, size, and shape of germ apertures; and the finer wall marking such as reticulations, perforations, etc. There is a growing interest in these morphological characters of pollen with reference to their taxonomic and phylogenetic significance. They are also the features by which pollen used by bees can be identified.

The observations on content were limited to starch, sugar, protein, and fats, substances of first importance in the diet of bees.

The papers on pollen morphology with reference to its diagnostic value are numerous. The reader is referred to Wodehouse (1928) and Tammes (1930) for brief reviews of a number of the more pertinent articles.

It is now generally recognized that the shape, and size of pollen and the structural features of the wall are indicative of taxonomic and phylogenetic relationships. In many cases the morphological features of most diagnostic value are the furrows, pores, spines, ridges, and other configurations of the pollen wall.

The *pollen wall* is generally described as two layered. Nearly a century ago Fritzsche introduced the terms "exine" and "intine" to designate these layers. The exine, or outer layer, when treated with certain reagents, appears to be composed of an outer and an inner region differing considerably in reaction. Kerner and Oliver called the outer portion of the exine "perine" and the inner portion "extine." Pope (1925) and Hayden (1930) use the term extine to designate both portions of the exine. Although there is considerable confusion in terms, exine and intine are used by most authors to designate respectively the outer and inner layers of the pollen wall.

The outer portion of exine forms a thin inconspicuous covering which is described by some authors as continuous and by others as discontinuous over the germ pores. The inner portion of exine is much greater but less uniform in thickness and is eutinizied. It is much reduced in thickness or entirely absent at the germ pores. The intine varies in thickness in the pollen of different plants, but is usually comparatively thin, except at the germ pores where it undergoes thickenings that occupy the openings in the exine. The outer portion of the intine was found by Mangin, Biourge, and others to consist chiefly of pectic substances. In the thickenings at the germ pores pectic substances are especially prominent. According to Kerner and Oliver the perine, or outer portion of exine, bears the sculpturings but other reports show that configurations may be borne by the inner portion of exine and also by the intine.

The *furrows* or *expansion folds* are narrow elongated areas in which

the exine is thinner and adornments either absent or less prominent. They are regarded as provisions for the folding of the wall in response to the shrinkage of the protoplasm through loss of water. The furrows usually encompass the germ pores and when they fold in the germ pores are closed over by the edges of the folds. Thus, by reducing the surface of the pollen and protecting the pores against evaporation, the furrows perform an important function in protecting the protoplasm against injury from drying. When shrivelled pollen comes in contact with moisture, the protoplasm quickly expands and forces out the folds to a convexity that conforms to the rest of the surface of the grain. In some articles on pollen the movements of the furrows are largely attributed to the shrinking and swelling of the pectic substances in the intine which is described as being closely attached exteriorly to the exine and interiorly to the protoplasm. The opening of the germ pore, usually present in the middle of the furrow, is also attributed partly to the swelling of the pectic substances of the thickened intine. In their swollen condition they tend to bulge out through the pores and are also more easily forced through the pores by the pressure exerted by the protoplasm.

Pope (1925) adopts the following system of terms to describe the surface of pollen grains:

Wall smooth—no characteristic markings.

Wall reticulate—thickenings on the extine, usually ridges that anastomose and form various patterns.

Wall punctate—many pit-like depressions.

Wall echinate—spines projecting from the surface.

Wall with pores—thin places in extine where pollen tubes protrude.

As to the shape she describes pollen as an elongated ellipse, spherical, dumbbell-shaped, ellipsoidal, cylindrical, pyramidal, and polyhedral. In her pollen studies, which covered numerous genera and about 80 families of the Colorado flora, Pope found shape and size of pollen grains in general corresponding rather closely in genera of same family. In some families, however, there were some striking differences. Her observations were made on dry pollen.

Wodehouse (1928, 1929, 1930, 1931 and 1932) reports extensive and detailed studies of pollen morphology with reference to its taxonomic and phylogenetic value. His articles contribute much additional evidence of the diagnostic value of pollen characters, and able discussions of the origin and relative diagnostic value of different pollen features. He introduces (1928) many descriptive terms new to the literature on pollen.

Those applicable to the pollen of the Leguminosae are as follows:

Expansion folds—longitudinal thin-walled areas, serving to accommodate changes in volume.

Germinal furrows—elongated, narrow areas in which germinal apertures are located and which differ from the remainder of the pollen-surface generally in the unadorned and thin character of the exine. Usually expansion folds are germinal furrows.

Tricolpate—characterized by three furrows or expansion folds.

Psilate—unadorned—without ridges, spines, or projections other than germ pores.

Lophate—with outer surface thrown into ridges, anastomosing or free.

Sublophate—ridges or crests inconspicuous or imperfectly defined.

Lacunae—areas bounded by the crests in lophate pollen.

Wodehouse (1929) makes two groups of pollen characters, those specifically inherited and those due to contact or other relations with their neighbors. The former, called *emphytic* and considered of much diagnostic value, are the spines, ridges, and other finer markings of the wall. The latter group, called *haptotypic*, and considered of little diagnostic value are the number and arrangement of germ furrows and apertures. The number and arrangement of these structures he thinks are determined mainly by the arrangement of the pollen grains in the tetrad during their development. For example, he ascribes the prevailing tricolpate condition of the pollen of Dicotyledons to the tetrahedral arrangement of the pollen in the tetrad stage. The tetrahedral arrangement provides each daughter cell of the tetrad with three points of contact each of which determines the location of a furrow and aperture. Points of contact and tendency toward symmetrical development he thinks are sufficient to account for all the differences in numbers and arrangement of furrows and apertures.

Tammes (1930) holds with Wodehouse that the number and arrangement of germ apertures or places of exit, as he calls them, are not strictly hereditary features. In some cases he found a close correlation between the size of grain and the number of places of exit. He states that in cases of polyploidy and abnormal reduction-division a great variation in number of places of exit may occur. In these cases the increase or decrease in number of chromosomes appears to run parallel with variations in size of grain and number of places of exit.

The interest in the content of pollen has been mainly with reference to hay fever, nutrition of bees, and the functioning of the pollen in fertilization. Most attention has been given to the starch, sugar, protein, and fat content of pollen.

Paton (1921), the first investigator to give much attention to pollen enzymes, identified eleven different enzymes in the eighteen species of pollen examined. Anderson's (1923) analysis of corn pollen shows a wide range in both minerals and organic constituents.

Sipe (1923) examined the pollen of 67 species distributed among 26 families. Starch was present in 14, sugar in 4, fat in 36, and protein in 35 of the 67 species. He found three germ pores usually present and shapes of pollen spherical, ellipsoidal, cylindrical, five-sided, and triangular. Range in diameter runs from 21 to 130 microns. He made his measurements in water mounts. He reports protein present in the pollen of four of the seven legumes included.

Hoffman (1925) gives the diameter and describes the shape and surface markings of four species from the genus *Trifolium*, two from the genus *Melilotus*, two from the genus *Medicago*, and one from the genus *Baptisia*. He mounted in both water and honey. In water their shape was spherical, subspherical or ellipsoidal. He notes the distinct reticulations on pollen of *Trifolium pratense* but not on the other species.

According to Hayden and Martin (1930) starch is generally present in abundance in pollen of vernal flowering trees. Martin found it present in all of the 15 species studied. Protein was also present in 12 of the 15 species. Hayden (1930) in another paper describes the pollen of *Melilotus alba*, *M. officinalis*, *Phaseolus multiflorus*, *Trifolium agrarium*, and *T. repens*. In each there are three germ pores and in all, excepting *Phaseolus*

multiflorus, the pores are described with lids. No surface configurations are mentioned other than germ pores.

MATERIAL AND METHODS

The pollen in all cases was taken from freshly opened flowers and from anthers shortly before or soon after they had dehisced. The object was to examine the pollen at the stage of maturity in which it reaches the stigma or is carried away to storage by bees. Pollen grains, especially in respect to content, commonly undergo marked changes during the late stages of their development. The most conspicuous of these changes is the disappearance of the starch which is so commonly abundant in the pollen of most plants a few days before dehiscence.

Immature pollen grains of most, if not all, of the species of Leguminosae examined contain starch which usually disappears before the pollen is dehisced. Consequently, comparisons of pollen grains in respect to content are not of much value unless the pollen grains compared are similar in stages of development.

Another very important factor that must be considered in examining pollen is the mounting medium. When pollen is exposed to the air it loses water rapidly and in thin-walled pollen the wall folds inward and the shape and size of the pollen are decidedly changed. Pollen that is spherical when turgid becomes ellipsoidal when shrivelled. The diameter parallel with the folds lengthens while the diameter at right angles to the folds shortens. The shape and dimensions of pollen are comparable only when they pertain to pollen in similar states of expansion. This makes the question of the mounting medium an important one.

There is a difference of opinion as to what should be regarded as the natural or normal condition of the pollen, the shrivelled or turgid condition. Pope (1925), Ferguson (1932), and a number of others made their observations on dry pollen, considering the shrunken condition more nearly natural than the turgid state. Wodehouse (1926) reports that the pollen of some Compositae are shrunken when shed from the anther and is inclined to regard neither condition as more nearly natural than the other. He found the turgid condition more favorable for revealing surface markings. He states that pollen grains should be observed in both dry and moist conditions but that the detailed studies should always be made on moist and fully expanded grains. Sipe (1923), Hoffman (1925), Hayden (1930) and others used water alone or as one among other mounting media.

In this discussion descriptions of shape and measurements pertain to fully expanded pollen. The expanded turgid condition is characteristic of pollen when it is functioning on the stigma and of active cells in general. In view of this fact the turgid condition is regarded as the more nearly normal one. For the study of shape and size the pollen was mounted in distilled water. The pollen of all the species responded quickly and became fully expanded in less than a minute and usually in a few seconds.

Another feature that has a bearing upon the reliability of measurements pertains to the stretching of the exine. If the exine is elastic, dimensions of pollen will vary somewhat with the length of time the pollen is permitted to absorb water. To obtain data pertaining to this question, measurements were made of pollen on stigmas and at intervals over a period of time in water mounts. Freshly pollinated stigmas of *Trifolium pratense*, *Medicago sativa*, and *Mcililotus alba* were mounted dry and the turgid pollen measured. The dimensions were in accord with those ob-

tained on water mounts. Measurements made in water mounts at intervals over a period of three minutes showed that the pollen attains a constancy in size soon after moisture is available, usually in a few seconds. These facts show that the exine is inelastic and consequently size of pollen does not vary to any extent after a certain degree of expansion is attained.

The diameters were measured with an ocular micrometer. The measurements were so made as to exclude the bulging at germ pores due to the high turgor pressure the protoplasm attains in water. The size of grains of each species is recorded in terms of range and greatest frequency, both of which are based upon fifty to several hundred measurements. In a number of species the estimates are based upon measurements made through a number of years. Both the ranges and frequencies are only roughly approximated. The comparatively wide range of variation in size of pollen in all the species, as the table shows, makes it difficult to arrive at statements of size that are reliably descriptive of the pollen of each species. The dimensions are of value where differences are large enough that there is little overlapping of dimension between species. The statement of range is somewhat misleading, because in most species there is a wide variation only when the few extremes are taken into consideration. The majority of pollen in most species range in size closely around the greatest frequency recorded. In two species, where there are two groups of pollen as to size, range, and frequencies for each group were estimated.

The furrows, germ pores, ridges of the reticulations and other surface markings were sufficiently distinct after treating the pollen with twenty per cent sodium hydroxide followed by methylene blue. Nigrosin was used occasionally for the staining of germ pores. The pollen was imbedded, either in gum arabic or paraffin, and sectioned when sectional views of the wall were required.

In identifying the substances in the content of the pollen the following reagents were used in making the tests: IKI for starch; Fluckiger's reagent for sugar; the Biuret reaction, eosin, and Millon's reagent for protein; and Sudan III for fat-like substances.

DESCRIPTION OF THE POLLEN OF THE LEGUMINOSAE SHAPE, SIZE AND STRUCTURAL FEATURES

Shape. The prevailing shapes of pollen in the 41 species of Leguminosae are globose and spherical. In the genera *Vicia*, *Melilotus*, *Petalostemum* and *Pisum* the pollen is ellipsoidal. The ellipsoidal shape is most pronounced in the genera *Vicia* and *Petalostemum*. In *Gymnocladus dioica* and *Gleditsia triacanthos* the pollen is noticeably flattened. Although there is considerable variation in shape even in the same anther, distinctive shapes, like ellipsoidal and spherical, are quite constant and decidedly characteristic of the species where they occur.

Size. The pollen varies from a greatest frequency of 18 to 72 microns in the 41 species. Small pollen is particularly characteristic of *Baptisia australis* and *Swainsona galegifolia*. Large pollen is characteristic of *Desmodium canadense*, *Apios tuberosa*, *Dolichos Lablab*, *Phaseolus vulgaris*, and *Strophostyles helvola*. In the majority of the species the greatest frequency in diameter is between 35 and 55 microns. In range of dimensions there is much overlapping between species. Only in a few cases, as in *Baptisia australis* and *Swainsonia galegifolia*, are the dimensions distinctive. In a number of species two types of pollen, large and small, are

present. This is particularly noticeable in *Desmodium canadense*, and *D. grandiflorum*.

Thickness of wall. The pollen of all species examined, excepting *Apios tuberosa*, have thin walls—in most species not more than one micron in thickness. In *Apios tuberosa* the thickness of the wall is near five microns or close to five times the thickness of the wall in other species, thus being a notable exception. Pope (1925), in a general discussion of pollen walls, states that all spherical pollen grains have exines more than three microns thick and in most cases eight to ten microns thick. When compared with these figures it is evident that the pollen of the Leguminosae investigated, with one exception, is characterized by exceptionally thin walls. The thinness of the wall accounts for its flexibility and the ease with which it folds or expands when the pollen loses or gains moisture.

Layers. The pollen-wall in all the species examined is two-layered, consisting of exine and intine as shown in text figure 1. The exine has an outer thin portion, previously referred to as perine, which constitutes a continuous covering over the grain. When broken at germ pores by the swelling of the thickened intine, fragments are often visible clinging to the edge of the pore. The inner portion of the exine is much thicker in most places than the outer layer. It is considerably reduced in thickness in the furrows and is omitted at the germ pores, leaving an opening into which the thickened intine intrudes. In sectional views of the pollen-wall of *Trifolium pratense*, where the reticulations are most prominent, the ridges show plainly as elevations of the exine. The intine is relatively thin except at germ pores. Here it is thickened with additional pectic substances.

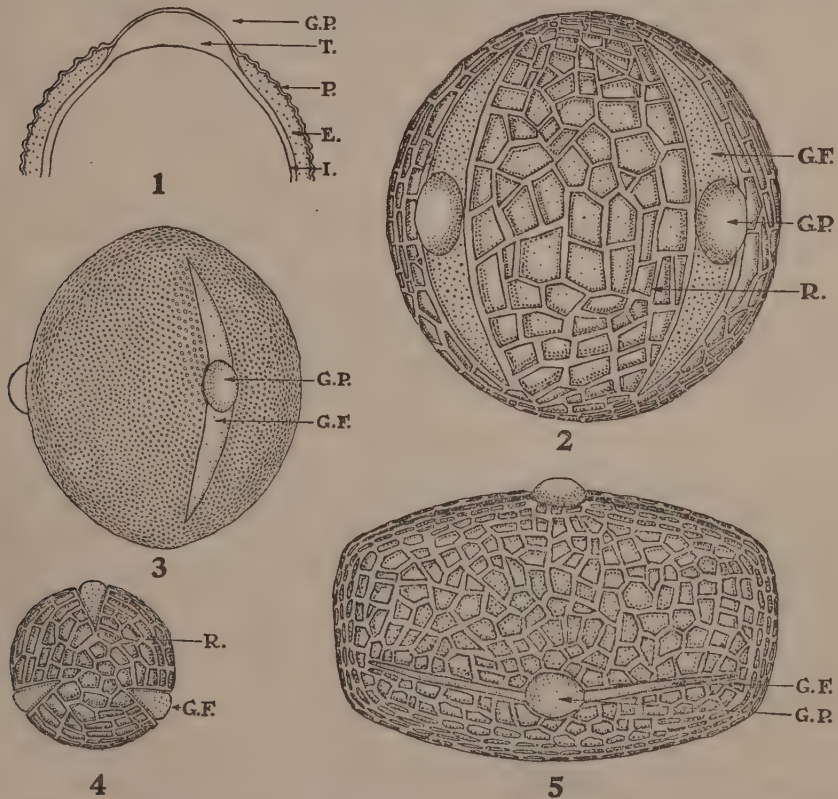
Three *germinal furrows* characterize the pollen of all the species. They appear as elongated, narrow, smooth areas differing in length and width in different species. In some species, notably in *Petalostemum purpureum*, *Vicia Americana*, and *V. villosa*, the furrows are very narrow (text fig. 5). In some species, as in *Trifolium pratense* and *Melilotus alba*, the furrows are almost as wide as the germ pore (text figs. 2 and 3). Each furrow midway of its length contains a germ pore. The furrows are equally distant from each other and extend at right angles to the circumference upon which the germ pores are located. In *Trifolium pratense* (text fig. 2) a high magnification shows that they are delimited by definite margins. In none of the species do they extend the full length of the grain but leave at the ends of the pollen an uninvaded area variable in size according to length of furrows and recognizable by the uniformity in appearance of its surface (text fig. 4).

In all these characters of the furrows, as shown by the limited observations, the pollen of most species show remarkable constancy. The most irregularity was noted in the genus, *Phaseolus*, where in some mounts many pollen grains had no furrows or very obscure ones. Pollen characterized by three furrows, a persistent feature throughout the 41 species, is described as *tricolpate* pollen by Wodehouse (1928).

The *germ pores* are uniform in structure throughout the species. They are simple in type; possessing no lids, plugs, etc. (text fig. 1). In some species they are comparatively large and in others small. They are pre-vaillingly circular but elliptical, rectangular, and much elongated shapes were observed in different species. The number of germ pores is almost invariably three throughout the species observed. In *Amphicarpa monoica*,

the only exception noted, three is the prevailing number of pores, but higher numbers are common.

Reticulations characterize the surface of the pollen not occupied by the furrows. They consist of a system of anastomosing ridges that divide the surface into areas or lacunae. The ridges vary much in height in different species. In *Trifolium pratense* they are so prominent as to be seen with low magnification when properly stained, but in most species a high magnification is required (text fig. 2). The distinctness of the reticulations depends much upon the height of the ridges. When the ridges are only very slight elevations then the reticulations are obscure. In a few species,



Figs. 1-5. 1, Sectional view through the wall of a pollen grain of *Trifolium pratense*, showing structural features typical of leguminous pollen. Wall is two-layered, consisting of intine (*I*) and exine, and latter being composed of an inner cutinized portion (*E*) interrupted at the germ pores, and an outer thin non-cutinized layer (*P*) that is continuous over the germ pores. The germ pore (*G.P.*) consists of opening in inner layer of exine, a lens-shaped thickened portion of intine occupying the opening in the exine, and the outer portion of exine covering the pore. 2, surface view of pollen of *Trifolium pratense*, showing the wide furrows (*G.F.*) and prominent reticulations (*R.*) on portions of the wall not occupied by furrows. 3, surface view of pollen of *Melilotus alba* showing wide germ furrow (*G.F.*) and lacunae of reticulation pore-like in size. 4, polar view of a pollen grain of *Trifolium pratense* showing the three germ pores (*G.P.*) and the uninvaded areas at poles, two features characteristic of leguminous pollen. 5, pollen of *Viola americana* showing the narrow furrows (*G.F.*).

in which pollen is described as smooth, they are exceedingly obscure or absent.

The lacunae vary much in size and shape in different species. In most species they are angular, ranging from triangles to polygons of many sides with triangles, rectangles, octagons, and hexagons prevailing. When the lacunae are exceedingly small, as in *Melilotus alba* (text fig. 3), they are pore-like in appearance and the wall is described as perforated or punctate. All gradations from large angular lacunae, as those characteristic of the pollen of *Trifolium pratense*, down to pores so fine that they are barely visible were observed in these 41 species of Leguminosae.

The general presence of reticulations and their variation in prominence throughout the species suggest that in the few species with apparently smooth walls, the reticulations may be only reduced below visibility.

A characteristic feature of the ridges is their smoothness. In *Petalostemum purpureum* the ridges of some of the pollen grains are sparsely adorned with short blunt spines and in the genus *Phaseolus* there is branching of walls and incomplete anastomosing. In all other species the ridges are not crowned with adornments of any kind and form smooth walls about the lacunae.

The reticulations, despite their variations in their many features, are fairly constant however in range and type of variations in the different species.

This type of reticulation, viz., with ridges bearing no spines or other adornments, Wodehouse (1928) calls *psilophate*. According to his terminology the species of Leguminosae mentioned in this discussion are characterized by a *tricolpate psilophate* pollen. The pollen of *Trifolium pratense* is illustrative of this type (text figs. 2 and 4).

CONTENT

Starch was found abundant in eight species and in slight amounts in a few other species. In some cases it is generally distributed among the grains but more commonly it is abundant in some grains and absent in others. The small pollen grains from an anther are more apt to contain starch—probably an indication of immaturity. In general the ripe pollen of these species is characterized by an absence of starch.

Sugar was found only in traces in a few species, and has no importance as an element of diet.

Protein is present in considerable amounts in the pollen of all species except in that of *Gymnocladus dioica*. These species of the Leguminosae are characterized by the dominance of protein in their pollen.

Fat-like substances are present in the pollen of a number of genera. In the genera *Trifolium* and *Medicago* where they are most prominent they may be an important element in the diet of bees.

DISCUSSION

This investigation included 26 of the 51 genera of Leguminosae given in Gray's Manual. In most of the genera, the pollen of only one species was studied and in no genus more than three. The view presented is, therefore, little more than a cross-sectional view of half the genera of the Leguminosae. The pollen characters of genera not included and the variations

TABLE OF DESCRIPTIONS OF POLLEN*

Name of Plant	Shape	Diameter in microns		Content				Surface	
		Range	Greatest frequency	Starch	Sugar	Protein	Fat	Furrows and germ pores	Finer wall markings
CAESALPINIOIDEAE									
<i>Gymnocladus dioica</i> Koch	biscuit	greatest diameter 30-50	40	much	none	none	none	3	reticulations indistinct
<i>Gleditsia triacanthos</i> L.	lens	longest diameter 30-45	40	much in small grains	"	some	some	3	finely reticulated
<i>Cassia Chamaecrista</i> L.	spherical	33-50	43	none	"	"	none	furrows long	"
<i>C. marilandica</i> L.	"	40-60	50-60	much	trace	"	"	furrows short	finely pitted
<i>Cercis canadensis</i> L.	slightly angled	24-30	28	"	some	present	"	3	finely pitted
PAPILIONOIDEAE									
<i>Baptisia leucantha</i> T. & G.	spherical	20-28	25	none	none	much	"	furrows short	smooth
<i>B. australis</i> R. Br.	"	15-22	18	"	"	"	"	3	finely reticulated
<i>Crotalaria spectabilis</i> Roth	nearly spherical	30-35 to 40x45	32x43	"	"	"	"	3	pitted
<i>C. retusa</i> L.	"	37x40 to 40x45	38x42	"	"	some	"	3	"
<i>Lupinus Hartwegii</i> Lindl.	slightly lobed	35-45	38	"	"	much	"	3	reticulate
<i>Trifolium pratense</i> L.	nearly spherical	longest diameter 40-60	48x50	"	trace	"	some	3	reticulations prominent

Table of descriptions of pollen* (continued).

Name of Plant	Shape	Diameter in microns		Content				Surface	
		Range	Greatest frequency	Starch	Sugar	Protein	Fat	Furrows and germ pores	Finer wall markings
<i>T. hybridum</i> L.	ellipsoidal	21x27 to 33x38	27x33	none	trace	much	some	3	finely reticulated
<i>T. repens</i> L.	nearly spherical	32x33 to 38x39	36x37	"	"	"	none	3	"
<i>Medilotus alba</i> Desr.	ellipsoidal	24x30 to 28x35	27x32	"	none	some	"	3	"
<i>M. officinalis</i> L.	"	24x28 to 28x36	28x34	"	"	"	"	3	"
<i>Medicago sativa</i> L.	spherical	31-44	36	"	"	much	"	3	indistinctly reticulated
<i>M. lupulina</i> L.	flattened to spherical		24x30	"	some	some	some	3	"
<i>Psoralea argophylla</i> Pursh.	spherical	20-50	40	"	"	"	"	3	reticulated
<i>Amorpha canescens</i> Pursh.	"	44-50	47	much	none	"	none	furrows long	pitted
<i>A. fruticosa</i> L.	"	21-50	40-45	none	"	"	"	3	smooth
<i>Petalostemum purpureum</i> (Vent.) Rydb.	ellipsoidal		33x50	"	"	"	some	3	ridges with spines
<i>Robinia Pseudo-Acacia</i> L.	nearly spherical	23-32	30	"	"	"	trace	3	smooth
<i>Astragalus canadensis</i> L.	slightly flattened	23x27 to 33x45	24x32	"	"	"	none	3	"
<i>Desmodium canadense</i> L. D. C.	3 angled and spherical	45-50 to 55-70	of large pollen 65	much	trace	"	"	3	"
<i>D. grandiflorum</i> (Walt.) D. C.	spherical	2 sizes	33 and 45	some	none	"	"	3	finely reticulated

Table of descriptions of pollen* (continued).

Name of Plant	Shape	Diameter in microns		Content				Surface	
		Range	Greatest frequency	Starch	Sugar	Protein	Fat	Furrows and germ pores	Finer wall markings
<i>Lespedeza striata</i> (Thumb.) H. & A.	nearly spherical	35-45	40	much	none	some	none	3	finely reticulated
<i>L. capitata</i> Michx.	spherical	27-47	40	none	"	much	some	3	pitted
<i>Vicia villosa</i> Roth.	ellipsoidal	27x45 to 33x55	30x48	"	"	"	none	3	finely reticulated
<i>V. americana</i> Muhl.	"	27x45 to 33x55	30x48	"	trace	"	"	3	"
<i>Pisum sativum</i> L.	"		30x38	"	none	"	"	3	"
<i>Lathyrus odoratus</i> L.	spherical	36-54	50	"	trace	"	"	3 furrows long and narrow	reticulated uniform lacunae
<i>Phaseolus coccineus</i> L.	"	45-57	54	much	"	trace	"	3	reticulated lacunae irregular
<i>P. vulgaris</i> L.	"	45-55	45	some	none	some	"	3	"
<i>P. timensis</i> Macf.	nearly spherical	35-50	43	much	"	much	"	3	"
<i>Strophostyles helvola</i> L.	spherical	42-57	50	none	"	some	"	3 furrows short	"
<i>Apios tuberosa</i> Moench.	"	50-72	60	"	trace	much	"	3	pitted
<i>Glycine max</i> Merr.	"	24-27	25	"	none	some	"	3	reticulations obscure
<i>Swinsona galegifolia</i> R. Br.	"	12-21	18	"	"	"	"	3	finely reticulated
<i>Dolichos Lablab</i> L.	"	45-60	50-54	some	some	much	"	furrows obscure usually 3	"
<i>Amphicarpa monoica</i> L. Ell.	"	24-50	36	none	none	"	"		pitted

* Sequence and names of species follow Gray's Manual, 7th Edition, and Bailey's Manual of Cultivated Plants.

in pollen features among the species of all the genera remain to be investigated. In the genera so far covered there are a number of persistent pollen characters that may have some significance.

The tricolpate character, so characteristic of the pollen of the Leguminosae described in this article, is common to many other families. Fischer alone reported it in about 1350 species of Dicotyledons. In accounting for the number and arrangement of furrows and germ pores both Wodehouse (1929) and Tammes (1930) go back to meiosis and attempt to show that these features are determined by the direction of the nuclear divisions and the method of wall formation.

Tammes illustrates nine types of arrangements the members of a tetrad may have. When no walls are formed until after the homeotypic divisions are performed and the two homeotypic divisions are at right angles to each other, the members of the tetrad have the tetrahedral arrangement and three points of contact. The reports of Farr (1915) and other cytologists show that the method of quadripartition by furrowing and the tetrahedral arrangement of the tetrad members are common in the Dicotyledons. In the genera, *Lathyrus*, *Trifolium*, *Medicago*, and *Melilotus* of the Leguminosae, meiosis has been followed and found to be of this type.

The tetrahedral arrangement, in providing the three points of contact between the members of the tetrad, insures the tricolpate character, for according to Wodehouse and Tammes each contact point or area locates a furrow and germ pore. Among other evidences which support his explanation, Wodehouse refers to the Ericaceae where the tetrads remain firmly united. Here the three furrows of each member are contiguous and continuous with those of its three neighbors. Also at the center of contact each of the furrows contains a germ pore. If this explanation of the tricolpate character is accepted, it follows that there is a remarkable uniformity in type of meiosis throughout the 41 species of Leguminosae.

A direct correlation between size, number of chromosomes, and number of germ pores has been reported in a number of plants. In the genus *Fuchsia* species with 22 chromosomes produce pollen with two germ pores, while species with 44 chromosomes produce pollen with three or four germ pores. Similarly in the genera *Solanum*, *Oenothera*, *Epilobium* and others where there are variations in number of chromosomes with accompanying differences in size of pollen, the tendency of the number of germ pores to vary with size of pollen has been reported. Tammes concludes from his investigations that the proportion between size of pollen and the number of places of exit is genetically determined and that a great variation in the number of places of exit especially comes about in such cases where factors are present which greatly influence the size of the pollen, as for instance a change in the number of chromosomes or a difference in size of pollen in heterostylism.

In the Leguminosae included in this investigation, no tendency in number of furrows or germ pores to vary with size of pollen or number of chromosomes was observed. The variations in diameter of greatest frequency range from 18 to 72 microns in the different species. Through this wide range in size of pollen the number of furrows and germ pores remains constant. In the same species where the range in size in some cases is quite marked, as for instance, in the genus *Desmodium* where there are two distinct sizes of pollen, the number of furrows and germ pores is constant.

The number of chromosomes in a number of the species is known. They range from 12 in the genus *Cassia* to 44-46 in the genus *Lupinus*.

The diploid numbers most common are 14 and 16. In the genera *Vicia*, *Lathyrus*, and *Pisum* 14 prevails while in the genera *Trifolium*, *Medicago*, and *Melilotus* the diploid number is 16. In the species of *Phaseolus* 22 and in *Glycine Max* 38 are the diploid numbers. Thus it is seen that there is considerable range in number of chromosomes presented by the different species with no apparent influence upon the number of furrows or germ pores as reported in some genera of other families. The persistence of the tricolpate character through a wide range in size and number of chromosomes of the pollen of the Leguminosae favors the view that this character is haptotypic, depending upon the influences the members of the tetrads exert upon each other.

The finer wall markings which are the characters considered of most diagnostic value are of the reticulate type with smooth ridges. In prominence of ridges, size and shape of lacunae, there are characteristic differences which may prove to be of much value in identifying genera and species. As the table shows the types of reticulations, as for instance pitted or finely reticulated, are common to a number of species and genera. The pollen grains of most of the species are distinguished only by a combination of all the pollen characters.

SUMMARY

The pollen grains of 41 species distributed among 26 genera of the Leguminosae are described as to morphological features and content.

The prevailing shape is globose to spherical.

The size in diameter ranges from 18 to 72 microns with the diameter of the majority falling within the range 35-55 microns.

The wall is two-layered, consisting of both exine and intine.

Germ pores are simple in type.

Three furrows equidistant and each containing a germ pore characterize the pollen of all the species.

The furrows run at right angles to the circumference upon which the germ pores are located.

There is no correlation between number and arrangement of furrows and the size or number of chromosomes of the pollen.

The characteristic finer markings are the reticulations which adorn the surface not occupied by the furrows.

In regard to the content protein or protein constituents are dominant.

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THE QUANTITATIVE CHEMICAL ESTIMATION OF TEXTILE FIBERS¹

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I. THE QUANTITATIVE ESTIMATION OF COTTON CELLULOSE IN THE PRESENCE OF WOOL, SILK, REGENERATED CELLULOSE RAYON, OR CELLULOSE ACETATE RAYON

The demand for definite information about the composition of union textiles increases the need for better methods of quantitative estimation for the different fibers of such mixtures. The methods which have been used for the estimation of cotton cellulose have chiefly been those by which all other fibers are dissolved and the residual cellulose is weighed. Variations in the use of a solvent as the size and preparation (61, 85, 88) of sample, concentration, time, and temperature of treatment have given rise to a range of corrections for the cellulose dissolved. A critical study of these methods has been made in an attempt to determine more definite values for the cellulose dissolved.

HISTORICAL

The effect of wool solvents on cotton

Dilute solutions of potassium hydroxide or sodium hydroxide have been used to dissolve the wool and occasionally the silk of textile mixtures. These methods are reviewed in table 1 and table 2.

TABLE 1. *The use of potassium hydroxide as a solvent for wool*

Method	Potassium hydroxide percentage	Time min.	Temperature	Loss in weight of cotton percentage
Stefanelli (127)	5		boiling	
Villavecchia and Hannau (135)	10		hot	
Dannerth (22)	5	15	boiling	5
Herzfeld (58)	10		boiling	
Darling (23)	5	10	boiling	5
Mackinnon (89)	5	2-4	boiling	
Harper (51)	10	10	boiling	5
Matthews (91)	5	20	boiling	3
Griffin (46)	5	10-15	boiling	3.5
A. S. T. M. (1)	5	10-15	boiling	3
Phelps (103)	5	10-20	boiling	5
Kauffman (74)	10	60	90°-100°C.	

¹ Journal Paper No. B63 of the Iowa Agricultural Experiment Station, Ames, Iowa.

^{2,3,4} Contributions by these writers were based on results reported in Master's theses. (See literature cited 157, 3, 37).

TABLE 2. *The use of sodium hydroxide as a solvent for wool*

Method	Sodium hydroxide percentage	Time min.	Temperature	Loss in weight of cotton percentage
Mulder (93)	concn.			
Bouchas (114)	5			
Stefanelli (127)	5		boiling	
Rémont (109)	2	15	boiling	5
Elsner (32)	1.5	15	boiling	5
Lossean (115)	1-2	10	boiling	
Richardson (111)	2	7	boiling	
Hunger (66)	10	15	boiling	4.0
	4	15	boiling	2.6
	2	15	boiling	2.3
Kapff (71), Pinagel (104)	2	15	boiling	3.5
Posselt (106)	5	15	boiling	5
Müller (94)	10	35	boiling	5
Duyk (30)	2	20	90°C.	
Green (44)	10	20	boiling	3.5
Bureau of Standards (16)	5	10	boiling	5
Grempe (45)	3			
Weltzien (153)	4	30	room	5.2
	6	30	room	5.7
Sommer (125)	4	30	boiling	3.5
Heermann (55)	10	35	boiling	3.5
Heermann (56)	2.5	15	boiling	3.5

The effect of silk solvents on cotton

Concentrated hydrochloric acid (93), alkaline copper or nickel hydroxides, basic zinc chloride, and concentrated aqueous solutions of calcium thiocyanate have been used as solvents for the silk of textile mixtures.

The use of hydrochloric acid as the differential solvent is summarized in table 3.

TABLE 3. *The use of concentrated hydrochloric acid as a solvent for silk*

Method	Time min.	Temperature	Loss in weight of cotton percentage
von Hönel (136)	0.5-2	boiling	
Richardson (111)	0.5	boiling	28
Posselt (106)	2-3.0	50°C.	
Collingwood (17)			4.1-5.0
Matthews (91)			4.0
A. S. T. M. (1)	2.0	49°C.	
Heermann (56)	3.0	boiling	

Schweizer (121, 122, 123) noted the solubility of silk fibroin in ammoniacal nickel hydroxide (41) and Stefanelli (127) and Persoz (102) used Schweizer's reagent to separate cotton, silk, and wool. Heermann and Sommer (57, 124) suggested the recovery of the cellulose from its solution (60) in Schweizer's reagent. However, it is Löwe's (87) alkaline copper glycerol solution which has been used generally to dissolve the silk of textile mixtures. The different methods are listed in table 4.

TABLE 4. *The use of alkaline copper glycerol as a solvent for silk*

Method	Time min.	Temperature	Loss in weight of cotton percentage
Richardson (111)	20	room	1.0-1.5
Posselt (106)	15	50°C.	
Dannerth (22)	30	room	
Villavecchia and Cappelli (134)	120-180	room	
Herzfeld (58)	10		

Schlossberger (117) described ammoniacal nickel hydroxide as a solvent for silk and stated that it had little effect upon cellulose. Richardson (111) proposed a quantitative separation of silk from mixtures by means of this solvent. Darling (23) and Duyk (30) have used similar solutions of nickel. The use of ammoniacal nickel hydroxide as a solvent for silk is presented in table 5.

TABLE 5. *The use of ammoniacal nickel hydroxide as a solvent for silk*

Method	Time min.	Temperature	Loss in weight of cotton percentage
Richardson (111)	2	room	0.45
	10	boiling	0.80
Posselt (106)	3	hot	1.2
Collingwood (17)			1.0-1.1
Dannerth (22)	5	20°C.	
Beucké and Collin (11)			1.5
Wadsworth (152)	10	room to near boiling	
Matthews (91)	2	room	1.0

Persoz (101) suggested basic zinc chloride as a solvent for silk. The ways this solvent has been used are listed in table 6.

TABLE 6. *The use of basic zinc chloride as a solvent for silk*

Method	Time min.	Temperature	Loss in weight of cotton percentage
Rémont (109)	2	boiling	
Richardson (111)	1	boiling	0.45-0.60
Collingwood (17)			1.3 -3.0
Harper (51)			1.5 -2.0
Matthews (91)	5	boiling	

von Weimarn (142, 143, 150, 144, 147, 145, 148) has shown that the concentrated aqueous solution of any readily soluble salt capable of strong hydration will disperse silk fibroin and Hönsch (64) has shown that cotton and wool are but slightly attacked by boiling solutions of calcium, lithium, potassium, or sodium chlorides or of potassium thiocyanate. Kraus and Markert (80, 81) have developed a method for the quantitative estimation of fibrous mixtures based upon the solubility of silk fibroin in a concen-

trated aqueous solution of calcium thiocyanate. They report a loss of from 2 to 4 per cent of cotton.

von Höhnel (136) dissolved the silk and wool of textile mixtures by a boiling half-saturated solution of chromic acid. Griffin (46) described cold half-saturated chromic acid as a rapid solvent for rayons, a slow solvent for silk, and as not affecting cotton and other plant fibers.

The effect of regenerated cellulose solvents on cotton

Regenerated cellulose rayon is soluble in such silk solvents as concentrated hydrochloric acid, basic zinc chloride, and boiling concentrated solutions of calcium thiocyanate and other readily soluble hydrated salts (137, 27, 59, 158, 159, 138, 139, 28, 141, 140, 151, 150, 146, 129, 149, 72, 73). Williams (158) found that a portion of the calcium thiocyanate might be replaced by a salt which does not react chemically with the thiocyanate, maintains its boiling point, has a high solubility in water, forms a viscous solution, and has a positive heat of dilution. He found that calcium chloride met these specifications.

Lloyd and Priestley (86) hydrolyzed the regenerated cellulose of mixtures by means of hydrochloric acid, sp. gr. 1.05, for 15 minutes at 50° C. or by sulfuric acid, sp. gr. 1.345, at 50° C. for 20 minutes.

The effect of cellulose acetate rayon solvents on cotton

The work of Schweiger (120) and von Weimarn (147) suggests the solution of cellulose acetate rayon by salts of the alkali and alkaline earth metals. Seventy per cent acetic acid has been suggested (132) as a solvent for cellulose acetate rayon in mixtures with wool. Lloyd and Priestley (86) used acetone at room temperature to dissolve the cellulose acetate rayon in dyed and undyed mixtures of this fiber and cotton, wool, or viscose rayon.

EXPERIMENTAL

The sample of fibrous material was prepared for analysis by boiling approximately five grams (samples of one gram were used with the calcium salts) of a plain-woven fabric of bleached cotton, viscose rayon, cellulose acetate rayon, or wool or of degummed silk in 500 cc. of water for one hour, rinsing, drying, extracting the fabric with anhydrous ether for 18 hours (the equivalent of 5 liters of ether) in a Soxhlet extractor (Plate I) and then drying the sample to constant weight in a weighing bottle in an oven at from 105° to 110° C.

The sample was immersed (and constantly stirred in the solutions of calcium salts) in the solvent during the period of treatment and the beaker containing the reaction mixture was covered with a watch glass. The residual cellulose from the treatments with sodium hydroxide, alkaline copper glycerol, or basic zinc chloride was rinsed first in 300 cc. of water, then in 200 cc. of one per cent acetic acid, and finally in hot water. The cellulose from the treatments with Fehling's solution or ammoniacal nickel hydroxide was rinsed in one per cent hydrochloric acid. After all treatments with hydrochloric acid the residues were rinsed with water until the rinse gave no test for chloride. The residues from the treatment with sulfuric acid were rinsed with water, dilute ammonium hydroxide, and finally with water. The residues of cellulose from the treatment with calcium salts were rinsed in running water for 30 minutes. Those from the treatment with 70 per cent acetic acid were rinsed in hot water, those from aniline

were rinsed twice with aniline (b.p. 184°C.) and then with methanol until completely decolorized. In the case of acetone the samples were treated with three fresh portions of the solvent (b.p. 56°C.) of 300 cc. each, for 60, 30, and 30 minutes. The residues were dried first at room temperature and then at 105°C. as before. All weighings were made with tares.

The special solvents were prepared as follows:

1. Alkaline copper glycerol

A 10 per cent solution of sodium hydroxide was added to a mixture of 25 cc. of glycerol, sp. gr. 1.25, and 500 cc. of a 10 per cent solution of crystalline cupric sulfate until the precipitate formed just dissolved.

2. Ammoniacal nickel hydroxide

a) Richardson's (111) reagent

A dilute solution of sodium hydroxide was used to precipitate nickel hydroxide from 500 cc. of a 5 per cent solution of crystalline nickel sulfate. The nickel hydroxide was washed with water, rinsed into a flask with about 125 cc. of aqua ammonia, sp. gr. 0.90, and made up to a volume of 250 cc. with water.

b) Darling's (23) reagent

Nickel oxycarbonate was precipitated from a 5 per cent solution of crystalline nickel sulfate by a saturated solution of sodium carbonate. The mixture was well shaken, filtered, washed free from sulfate, and dissolved in a 20 per cent solution of ammonium hydroxide.

c) Fifty grams of crystalline nickel sulfate were dissolved in 160 cc. of boiling water; the solution was cooled and the nickel precipitated as hydroxide by 70 cc. of a 20 per cent solution of sodium hydroxide. This precipitate was washed with water four times by decantation, filtered by suction on a Büchner funnel, transferred to a flask, thoroughly mixed and made up to a volume of 500 cc. with aqua ammonia, sp. gr. 0.90. The freshly prepared reagent was mixed thoroughly before use.

3. Basic zinc chloride

One thousand parts of zinc chloride, 850 parts of water, and 40 parts of zinc oxide were mixed and heated until all the zinc oxide dissolved.

4. Fehling's solution

Equal volumes of solutions A and B were mixed just before use.

A. Three and five-tenths grams of crystalline cupric sulfate were dissolved in 100 cc. of water.

B. Seventeen grams of Rochelle salt were dissolved in 15 cc. of water; a solution of 5 grams of sodium hydroxide in 15 cc. of water was added and the mixture was cooled and diluted to 100 cc.

5. Solution of calcium thiocyanate

A 50 per cent solution of $\text{Ca}(\text{CNS})_2 \cdot 3\text{H}_2\text{O}$, boiling point 140°C. , was heated to 70°C. and filtered.

6. Solution of calcium thiocyanate and calcium chloride

A mixture of equal volumes of a 50 per cent solution of $\text{Ca}(\text{CNS})_2 \cdot 3\text{H}_2\text{O}$, boiling point 140°C. , and of a 50 per cent solution of CaCl_2 , boiling point 140°C. , was heated to 70°C. and filtered.

TABLE 7. *The solubility of cotton cellulose in dilute sodium hydroxide*

Sodium hydroxide normality	Volume cc.	Time min.	Temperature	Determinations number	Loss in weight of cotton cellulose		
					minimum percentage	mean percentage	maximum percentage
1.2500	500	20	90°C.	8	1.39	1.65	1.93
0.5028	500	15	boiling	10	2.96	3.33	3.97
0.5028	500	15	90°C.	7	1.15	1.32	1.47
0.2500	500	30	80°C.	6	1.55	1.73	1.92
0.1250	500	30	boiling	12	2.65	3.28	3.97

TABLE 8. *The solubility of cotton cellulose in solvents for silk fibroin and regenerated cellulose*

Solvent	Volume cc.	Time min.	Temperature	Determinations number	Loss of weight of cotton cellulose		
					minimum percentage	mean percentage	maximum percentage
Alkaline copper glycerol	300	15	50°C.	10	0.13	0.22	0.50
Basic zinc chloride	300	1	boiling	8	0.19	0.32	0.43
Calcium thiocyanate	100	60	100°C.	3	1.34	1.36	1.38
Calcium thiocyanate and calcium chloride	100	60	100°C.	3	5.08	5.61	6.46
Fehling's solution	200	3	boiling	11	0.21	0.38	0.58
Hydrochloric acid, sp. gr. 1.05	300	15	50°C.	6	0.51	0.78	0.97
Hydrochloric acid, sp. gr. 1.19	300	2	room	10	0.25	0.34	0.54
Sulfuric acid, sp. gr. 1.345	300	20	50°C.	5	0.77	1.01	1.29

TABLE 9. *The effect of solvents for cellulose acetate rayon on cotton cellulose*

Solvent	Volume cc.	Time min.	Temperature	Determinations number	Change in weight of cotton cellulose		
					minimum percentage	mean percentage	maximum percentage
Acetic acid, 70 per cent	300	90	room	5	-0.15	-0.20	-0.23
Acetone	300	120	room	4	0.00	0.00	0.00
Acetone, in the presence of 5 gm. of cellulose acetate rayon	300	120	room	6	-0.04	+0.17	+0.29
Aniline	300	15	90°C.	6	-0.02	-0.16	-0.20
Aniline, in the presence of 5 gm. of cellulose acetate rayon	300	15	90°C.	5	0.00	+0.10	+0.41

A half-saturated solution of chromic acid at room temperature rapidly dissolved the regenerated cellulose of a viscose rayon and cotton cellulose mixture but with the evolution of heat; the cotton was too much tendered to be recovered satisfactorily. At 10° C. the regenerated cellulose dissolved very slowly and the cotton was tendered beyond recovery.

Schweizer's reagent, prepared (79) by passing air for a day through copper turnings covered with aqua ammonia, although it dissolved the regenerated cellulose rapidly and completely, was of no use for a quantitative separation of regenerated cellulose and cotton cellulose because in the time required for solution of the former about 27 per cent of the latter dissolved.

Approximately 5 grams of silk dissolved to the extent of 0.95 per cent after 2 minutes at room temperature, and 10.37 per cent after 5 minutes at boiling temperature in 100 cc. of ammoniacal nickel hydroxide prepared according to Richardson; Darling's reagent was as ineffective. It was found that 5 grams of silk dissolved completely when boiled for 5 minutes in 350 cc. of the reagent prepared as in 2 c. However, when used for the quantitative analysis of textile mixtures, nickel hydroxide deposited on the fibrous residues, was difficult of removal, and gave inconsistent results. Cotton in ten determinations lost as much as 0.21 per cent or gained as much as 1.28 per cent; the corresponding percentages for cellulose acetate rayon in five determinations were 0.92 and 1.07 and for wool in seven determinations, 1.37 and 0.02.

The residues from the treatment with dilute sodium hydroxide did not check within ± 0.3 per cent as reported by Hedrick (53); the loss in weight of the cotton cellulose is given in table 7.

The loss in weight of cotton cellulose in the other solvents, alkaline copper glycerol, basic zinc chloride, calcium thioeyanate, Fehling's solution, and hydrochloric acid and by the two hydrolytic methods was lower than reported in the literature. The replacement of part of the calcium thioeyanate by calcium chloride appreciably increased the amount of cotton cellulose dissolved. Table 8 gives a résumé of the results.

The effects on cotton cellulose of three solvents for cellulose acetate rayon, 70 per cent acetic acid, acetone, and aniline, are given in table 9.

SUMMARY

1. A half-saturated solution of chromic acid has been shown ineffective in the quantitative separation of regenerated cellulose and cotton cellulose.

2. Schweizer's reagent has been shown ineffective in the quantitative separation of a mixture of regenerated cellulose and cotton cellulose.

3. It has been shown that silk fibroin does not dissolve completely in ammoniacal nickel hydroxide prepared according to directions given in the literature but that one gram of silk completely dissolves after 5 minutes at the boiling temperature in 70 cc. of ammoniacal nickel hydroxide prepared by using 425 cc. of aqua ammonia, sp. gr. 0.90, for a volume of 500 cc. Ammoniacal nickel hydroxide has been shown to be an unsatisfactory solvent for silk in the quantitative analysis of mixtures because the undissolved nickel hydroxide deposits on the fibrous residue and is extremely difficult of removal.

4. The solvent action for cotton cellulose has been studied in the case of the differential solvents used for the direct determination of the cellulose of textile mixtures, sodium hydroxide, hydrochloric acid, alkaline copper glycerol, basic zinc chloride, Fehling's solution, calcium thiocyanate, calcium thioeyanate and calcium chloride, 70 per cent acetic acid, acetone, and aniline.

5. Two hydrolytic methods have been studied for the separation of regenerated cellulose from cotton cellulose.

II. THE QUANTITATIVE ESTIMATION OF REGENERATED CELLULOSE IN THE PRESENCE OF COTTON CELLULOSE, WOOL, SILK, OR CELLULOSE ACETATE RAYON

The direct method (55) which has been used for the estimation of viscose rayon and cuprammonium rayon in textile mixtures has been that which makes use of alkali as a solvent for wool and silk. Since the regenerated celluloses are somewhat soluble in alkali under these conditions and moreover have usually been previously treated with alkali in the course of production the development of correction factors for the particular regenerated cellulose to be analyzed is essential.

Cross and Bevan (20) noted the solubility of hydrated celluloses in alkali, Hazard (52) and Gray, Staud, and Feuss (42) found that the extreme solubilities in alkali of cellulose and of regenerated cellulose were in the same region of concentration, and Weltzien (154) suggested the characterization of the esters of cellulose by a determination of the solubility in alkali of the cellulose regenerated from the ester. Jennings (69) reports a treatment of rayon crêpes for 30 minutes with 2 per cent sodium hydroxide at room temperature and Cosne (19) the optimum conditions for the desulfurization of viscose as 20 minutes at from 60° to 65° C. in from 0.8 to 1.0 per cent sodium hydroxide. A résumé of the solubility of regenerated celluloses in alkali is given in table 1.

EXPERIMENTAL

A bleached viscose rayon fabric of plain weave was prepared and studied in the same manner in which cotton was studied in part I. The temperatures were maintained by a water bath heated on an electric hot plate.

The effect upon regenerated cellulose of the solvents for wool, silk, and cellulose acetate rayon is given in table 2.

SUMMARY

The effect of dilute sodium hydroxide solutions, Fehling's solution, alkaline copper glycerol, and acetone on the weight of viscose rayon has been determined under the conditions in which these solvents are employed in the quantitative separation of textile mixtures.

III. THE QUANTITATIVE ESTIMATION OF SILK IN THE PRESENCE OF REGENERATED CELLULOSE OR CELLULOSE ACETATE RAYON

Two types of methods are available for the estimation of silk in mixtures with rayons. A proximate analysis based on a determination of the nitrogen content (38) depends on the constancy of the nitrogen of finished silks and is limited in its application to mixtures containing no other nitro-

TABLE 1. *The solubility of regenerated celluloses in alkali*

Method	Rayon	Solvent	Concentration percentage	Time min.	Temperature	Loss of weight percentage
Krais and Biltz (79)	cuprammonium viscose	sodium hydroxide "	5	15	82°-93°C.	6
Johnson (70)	cuprammonium viscose	sodium hydroxide "	5	15	82°-93°C.	7
Weltzien (154)	cuprammonium "	sodium hydroxide "	4	30	18°-19°C.	3.96
	viscose	"	6	30	18°-19°C.	2.26
	"	"	4	30	18°-19°C.	0.6
	"	"	6	30	18°-19°C.	1.3
	"	"	6	30	18°-19°C.	1.7-3.1
Ristenpart (113) Hall (47)	rayons viscose	sodium hydroxide "	4	30	18°-19°C.	4.0-6.3
	"	"	4	60	boiling room	2.27
	"	"	5	60	"	3.4
	"	"	6	60	"	4.5
	"	"	7	60	"	9.0
Hall (48)	"	"	8	60	"	12.5
	viscose	potassium hydroxide "	4	60	room	1.0
	"	"	8	60	"	1.4
	viscose	potassium hydroxide "	10	60	room	1.6
	"	"	15	60	"	1.8
Lloyd and Priestley (86)	"	"	20	60	"	2.5
	Chardonnet	potassium hydroxide or sodium hydroxide	1	60	boiling	13-14
	cuprammonium viscose	"	1	60	boiling	4-7
	"	"	1	60	boiling	6-10
	"	"	2.5	15	boiling	5-8

TABLE 2. *The solubility of regenerated cellulose in solvents for wool, silk and cellulose acetate rayon*

Solvent	Volume cc.	Time min.	Temperature	Determinations number	Loss of weight of regenerated cellulose		
					minimum percentage	mean percentage	maximum percentage
1.2500 N NaOH	500	20	90°C.	6	3.87	4.21	4.56
0.5000 N NaOH	500	15	boiling	6	2.87	3.04	3.24
0.5000 N NaOH	500	15	90°C.	6	1.90	2.13	2.35
0.2562 N NaOH	500	30	80°C.	6	1.31	1.43	1.59
Fehling's solution	200	3	boiling	5	1.14	1.18	1.21
Alkaline copper glycerol	300	15	50°C.	6	0.54	0.70	0.86
Acetone	300	90	room	5	+0.02	0.10	0.18

TABLE 1. *The effect of solvents for regenerated cellulose on cellulose acetate rayon upon silk fibroin*

Solvent	Volume cc.	Time min.	Temperature	Determinations number	Change in weight of fibroin		
					minimum percentage	mean percentage	maximum percentage
Acetic acid, in the presence of 5 grams of cellulose acetate rayon	300	20	to 85°C. room	2	-2.23	-2.95	-3.65
Acetone	300	120		2	+0.04	+0.05	+0.05
Acetone, in the presence of 5 grams of cellulose acetate rayon	300	120	room	9	-0.07	+0.18	+0.31
Aniline, in the presence of 5 grams of cellulose acetate rayon	300	15	90°C.	10	+1.20	+3.40	+6.18
Hydrochloric acid, sp. gr. 1.05	300	15	50°C.	6	-2.12	-2.45	-2.76
Sulfuric acid, sp. gr. 1.345	300	20	50°C.	5	-4.16	-4.72	-5.49

gen. In the second type of analysis the other fibers are dissolved and the silk residue is weighed.

EXPERIMENTAL

A plain-woven fabric of degummed silk was prepared and studied in the manner used for cotton in I.

The results of the study are given in table 1.

SUMMARY

1. Cellulose acetate rayon may be quantitatively separated from mixtures with silk by solution in acetone.

2. Neither 70 per cent acetic acid nor aniline are satisfactory for the quantitative separation of cellulose acetate rayon from silk.

3. For the quantitative separation of regenerated cellulose from silk the hydrolytic method using hydrochloric acid, sp. gr. 1.05, is preferable to that employing sulfuric acid, sp. gr. 1.345.

IV. THE QUANTITATIVE ESTIMATION OF WOOL IN THE PRESENCE OF COTTON CELLULOSE, SILK, REGENERATED CELLULOSE, OR CELLULOSE ACETATE RAYON

Two kinds of analysis may be used for the estimation of wool in union textiles. In the one all other fibers are dissolved and the residue of wool is weighed; the second is a proximate analysis based on a determination of the nitrogen or sulfur of wool. The proximate analysis depends on the constancy of the nitrogen or sulfur of finished wools (160) and is limited to mixtures containing no other nitrogen or sulfur.

The ways in which the differential solvents for cotton, regenerated cellulose, and silk have been used in the estimation of wool are summarized in table 1.

EXPERIMENTAL

A bleached wool fabric of plain weave was prepared and studied in the way in which cotton (I) was studied. The residual wool from the alkaline treatments was first rinsed in water, then in one per cent acetic acid, and finally in water, while that from the acid treatments was rinsed in water until the rinse gave no test for acid. About a gram of cellulose acetate rayon and a gram of wool were treated with 125 cc. of acetone for an hour; the solution was then decanted through a 240-mesh sieve and the residue of wool was stirred thoroughly in five portions of acetone (20 cc. each) for 30 minutes.

The effect of the various reagents on the wool keratin is given in table 2.

SUMMARY

1. The use of sulfuric acid, sp. gr. 1.524, for 20 minutes at 25° C. has been shown unsatisfactory as a differential solvent for cotton or regenerated cellulose in the quantitative estimation of wool.

2. The use of sulfuric acid, sp. gr. 1.345, for 24 hours at room temperature has been shown unsatisfactory as a differential solvent for cotton or regenerated cellulose in the quantitative estimation of wool.

3. The effect on the weight of wool keratin is reported for acetone, alkaline copper glycerol, basic zinc chloride, calcium thiocyanate, calcium thiocyanate and calcium chloride, hydrochloric acid, sp. gr. 1.19, hydrochloric acid, sp. gr. 1.05, Schweizer's reagent, and sulfuric acid, sp. gr. 1.345.

TABLE 1. *The effect of solvents for cotton, silk, regenerated cellulose, or cellulose acetate rayon on wool*

Solvent	Method	Time		Concentration percentage	Temperature	Loss of weight of wool percentage
		hr.	min.			
Acetic acid	(132)			70		
Acetone	Lloyd and Priestley (86)				boiling room	9.16
Alkaline copper glycerol	Richardson (111)		20		room	0.33
Ammoniacal nickel hydroxide	Richardson (111)		2		room	1.0-1.4
	Collingwood (17)		2		room	1.5
	Matthews (91)		1		boiling	1.43
Basic zinc chloride	Collingwood (17)		2		boiling	1.5-2.0
	Richardson (111)				boiling 100°C.	
	Duyk (30)	1				
Calcium thiocyanate	Krais and Markert (80, 81)			50		0.43-3.00
Hydrochloric acid	Collingwood (17)			concn.	50°C.	
	Posselt (106)		2-3	concn.	room	
	Matthews (91)		15	concn.	50°C.	
	Lloyd and Priestley (86)			10		
	Barreswill (4)			concn.		
Nitric acid	Krais and Biltz (79)	1				0.42
Schweizer's reagent	Bayer (7)	17		83	room	2
Sulfuric acid	Jawalowski (68)	12		75	room	
	Villavecchia and Hannan (135)	2		75	room	
	Dannerth (22)	12		80	room	
	Heerman (56)	6		80	room	1.5
	Horzfeld (58)	12		83	room	2
	Krais and Biltz (79)	24		44	room	
	Harper (51)	2		28	room	
	Matthews (91), Green (44)	12		60	room	2.5
	Griffin (46)		15	75	room	
	Lloyd and Priestley (86)		20	44	50°C.	

TABLE 2. *The effect of the differential solvents on wool keratin*

Solvent	Volume cc.	Time		Temperature	Determinations number	Change in weight of wool		
		hr.	min.			minimum percentage	mean percentage	maximum percentage
Acetone	125	1		room	4	+0.01	+0.04	+0.09
Alkaline copper glycerol	300		15	50°C.	4	+0.19	+0.38	+0.56
Basic zinc chloride	300		1	boiling	4	-0.11	-0.32	-0.47
Calcium thiocyanate	100	1		100°C.	4	-0.91	-0.92	-0.93
Calcium thiocyanate and calcium chloride	100	1		100°C.	6	-0.15	-0.39	-0.57
Hydrochloric acid, sp. gr. 1.19	100		2	25°C.	6	+0.06	+0.10	-0.44
Hydrochloric acid, sp. gr. 1.05	300		15	50°C.	5	-1.81	-1.84	-1.94
Schweizer's reagent	100		20	25°C.	6	-0.23	-0.50	-0.86
Sulfuric acid, sp. gr. 1.524	100		20	25°C.	6	-35.12	-35.84	-37.36
Sulfuric acid, sp. gr. 1.345	300		20	50°C.	6	-1.39	-1.51	-1.68
	300		24	heated to 50°C. and cooled at room temperature	4	-30.93	-31.68	-32.04

V. THE QUANTITATIVE ESTIMATION OF CELLULOSE ACETATE RAYON IN THE PRESENCE OF WOOL, SILK, OR CELLULOSE

An attempt has been made in this study to devise direct methods for the quantitative estimation of cellulose acetate rayon in the presence of wool, silk, or cellulose. Direct methods involving the recovery of the ester have been formulated from the indirect solution methods, and methods for the determination of the acetyl content of cellulose acetates have been adapted to the proximate analysis of the cellulose acetate rayon of union textiles.

HISTORICAL

Two indirect methods have been reported for the estimation of the cellulose acetate rayon of textile mixtures. An anonymous article (132) recommends boiling 70 per cent aqueous acetic acid as a solvent for cellulose acetate rayon in mixtures of this rayon with wool. Lloyd and Priestley (86) used acetone at room temperature to dissolve the cellulose acetate rayon of dyed and undyed mixtures of this fiber and cotton, viscose rayon, or wool. They found that this procedure gave low results and attributed the loss to a slight hydrolysis (50) of the ester in the preparation of the fabric (35) because the cellulose acetate obtained by dissection was not completely soluble in acetone and showed different dyeing properties.

The methods which have been used to determine the acetyl content of cellulose acetates may be divided into two groups, those of acid hydrolysis and those of alkaline hydrolysis.

Methods of acid hydrolysis

Green and Perkin (43) hydrolyzed 0.4 gram of cellulose triacetate in a mixture of 30 cc. of absolute alcohol and 2 cc. of sulfuric acid. The hydrolysate was slowly distilled and when the liquid in the distilling flask evaporated to half of its original volume more alcohol was added and this process was repeated three times. The distillate of ethyl acetate was collected in standard alcoholic potassium hydroxide and the alkali in excess of that required for saponification was titrated with a standard acid. The method yielded acetic acid values of 61.68, 61.36, 61.50, and 63.0 per cent (theoretical, 62.5 per cent). Fenton and Berry (33) repeated the method of Green and Perkin using phosphoric acid as the hydrolytic agent and obtained values of 52.2 and 54.4 per cent acetic acid from a cellulose acetate.

Ost (98) developed a method for the hydrolysis of cellulose acetate by 1:1 sulfuric acid or by 65 per cent phosphoric acid and reported an agreement among results within 1.5 and 2.0 per cent acetic acid. Ost and Katayama (100) reduced the time of hydrolysis from 48 to 24 hours and the volume of the distillate from 800 to 600 cc. and reported an error of ± 1 per cent acetic acid. Later Ost (99) gave the error of this method as 0.5 per cent acetic acid although Barnett (2) considered the error greater than 1 per cent. Berl and Smith (9), Schliemann (116), Beck (8), Böeseken, van der Berg, and Kerstjens (13), Oddo (96), Tatu (131) and Ridge, Parsons, and Corner (112) have reported the use of Ost's method; Schliemann gave an error of ± 1 per cent acetic acid, Böeseken and his coworkers obtained values of 62.5, 62.5, and 62.9 per cent acetic acid for a triacetate although they suggested that the concordance of the results might be accidental. Stein (128) showed that the production of volatile acid from the ester varied with the volume of the distillate rather than with the time

of distillation, changed Ost's procedure for the analysis of the esters of greatly modified celluloses, and corrected for other volatile acids produced by the ester. Fenton and Berry (33) obtained yields of 51.5 and 55.0 per cent acetic acid from a cellulose acetate by Ost and Katayama's method.

Phosphoric acid has frequently been recommended (82, 92) in place of sulfuric acid as the hydrolytic agent. This eliminates the error caused in some instances by the formation of sulfur dioxide during distillation. Hess and his coworkers (62, 63, 118) after Wenzel (155), introduced a buffered solution of phosphoric acid as the hydrolytic agent. This method of Hess is generally considered the most reliable (acetic acid values check within 0.1 to 0.3 per cent) macro method but it is a method impractical for general textile analysis. Billing and Tinsley (12) modified Ost's method in a somewhat similar way for use when both nitrogen and acetic acid are to be determined from the same sample; they obtained results within -0.19 and -0.84 per cent of the theoretical.

Toluene sulfonic acid has been used as the hydrolytic agent by Sudborough and Thomas (130) and in the micro methods of Freudenberg (39, 40, 14) and Weber (error of -0.1 to +0.4 per cent acetic acid) and of Pregl (107) and Soltys (error of ± 0.5 per cent acetic acid).

Deschiens (25) used 2:1 sulfuric acid in a modification of Ost's method and Krüger (83) and Tschirch 75 per cent sulfuric acid for 15 minutes.

All methods for acid hydrolysis of cellulose acetates are time-consuming and of doubtful accuracy because the large volume of the distillate renders the end-point indefinite and because volatile acids other than acetic are produced during the hydrolysis and distillation of the ester.

Methods of alkaline hydrolysis

Cross, Bevan, and Beadle (21) used boiling 0.5 N alcoholic sodium hydroxide as a saponifying agent for cellulose acetate; the excess of alkali they determined by titration with a standard acid. Green and Perkin (43) boiled 2 grams of dried (105° C.) cellulose acetate for 2 hours with this reagent and obtained values of 59.51, 61.24, and 61.33 per cent acetic acid. Fenton and Berry (33) obtained 58.3 and 61.9 per cent acetic acid by Green and Perkin's method. Ost (98) refluxed cellulose acetate with 0.5 N alcoholic potassium hydroxide and obtained 67.2, 67.8, and 62.51 and 61.4 and 63.2 per cent acetic acid. Mork (161) used a more dilute solution of boiling alcoholic alkali for an hour and Stadlinger (126) and Ohl (97) 0.5 N alcoholic potassium hydroxide for 48 hours at room temperature.

Woodbridge (161) modified Cross and Bevan's (162) sodium ethylate method for cellulose benzoate and hydrolyzed cellulose acetate 16 hours at room temperature in an excess of a solution prepared by dissolving 23 grams of sodium in 2 liters of 95 per cent alcohol.

Eberstadt (31) found that previous swelling of the acetate in equal parts of acetone and alcohol or of water and alcohol greatly hastened saponification. After swelling, the sample of about a gram was saponified by 100 cc. of 0.5 N potassium hydroxide in a stoppered flask at 25° C. over a period of 48 hours after which 50 cc. of the hydrolysate were titrated with N hydrochloric acid and the remainder of the hydrolysate was titrated with 0.5 N acid in the presence of phenolphthalein. Eberstadt made no correction for the moisture content of the ester.

Torii (133) modified Eberstadt's method; he added alcohol and then 10 cc. of N alkali to 0.2 or 0.3 gram of ester and after an hour at room

temperature the excess of alkali was titrated with N acid. Bernoulli, van der Berg, and Kerstjens (10) obtained 54.49 and 54.32 per cent of acetic acid by Yarsley's modification of Torii's method as compared with 54 ± 0.5 per cent acetic acid by Ost's method. Yarsley treated 0.5 gram of acetate with 2 cc. of alcohol, saponified it with 10 cc. of N sodium hydroxide for 75 minutes at room temperature, and titrated the excess of alkali first with N sulfuric acid and then with 0.1 N sulfuric acid in the presence of phenolphthalein.

Knoevenagel (75, 77) showed that 0.5 N potassium hydroxide at room temperature in a few hours quantitatively saponified cellulose acetate previously swollen in alcohol and that the degree of saponification varied as the degree of swelling. He proposed the following method (76) for which he gave an error of ± 0.5 per cent acetyl and Gutsche (82) an error of ± 1 per cent acetyl: One gram of cellulose acetate, dried at 100°C ., is treated with 20 cc. of 75 per cent alcohol for 30 minutes at from 50° to 60°C . before the addition of 50 cc. of 0.5 N potassium hydroxide. The mixture is heated to 50°C . and allowed to cool for 24 hours. The excess of alkali is then titrated with 0.5 N sulfuric acid in the presence of phenolphthalein.

Knoevenagel and König (78) reported values for acetic acid 0.8 per cent lower than Ost's by this method or of 53.5 as compared with 53.5 by Werner's (78) and 52.34 and 52.29 by Barnett's (2) method. Hess (62) claimed Knoevenagel's results were 2 per cent too high. Malm and Clarke (90), Fermazin (34) and Feuss and Staud (36) have reported the use of Knoevenagel's method; the last investigators obtained checks within 0.2 and 0.6 per cent acetic acid. Murray, Staud, and Gray (95) obtained acetyl values checking within 0.4 per cent by Knoevenagel's method.

Pringsheim, Leibowitz, Schreiber, and Kasten (108) hydrolyzed alcohol-treated cellulose acetate with 0.1 N sodium hydroxide for 24 hours at room temperature, titrated the excess of alkali with 0.1 N hydrochloric acid and obtained 42.0, 43.1, 42.9, 43.0, 42.8, 41.2, 43.3, and 43.1 as percentages of acetyl.

Barthélemy (5) hydrolyzed 2 grams of cellulose acetate with 40 cc. of N sodium hydroxide at 85° to 90°C . for 5 hours. After 9 to 16 hours more at room temperature the mixture was heated and then cooled and made up to a volume of 250 cc. Portions of 50 cc. each were titrated with 0.2 N sulfuric acid using phenolphthalein or Congo Red as the indicator. Barthélemy reported an error of ± 0.5 per cent acetic acid. Ost maintained the error was ± 2 per cent as was found by Fenton and Berry (33) although Deschiens (25) considered Barthélemy's method exact.

Böeseken and his coworkers (13) saponified one gram of cellulose triacetate with 10 cc. of 25 per cent potassium hydroxide for 48 hours at room temperature, added sulfuric acid in excess, titrated the excess of acid with barium hydroxide and obtained 61.1 per cent acetic acid. Fenton and Berry (33) obtained 53.5 per cent acetic acid by this method. Böeseken and his coworkers (13) also saponified one gram of cellulose acetate with aqua ammonia, sp. gr. 0.92, for 30 hours in a closed vessel at 100°C ., weighed the residual cellulose, and from the loss in weight of the ester upon hydrolysis calculated the acetic acid value as 58 per cent.

Irvine and Hirst (67) boiled 0.5 gram of the triacetate 1.5 hours with an excess of 0.5 N sodium hydroxide; they reported an acetyl value, corrected by a blank determination, of 44.2 (theoretical, 44.8).

Werner and Engelmann (156) developed a method for the analysis

of cellulose acetates which yield less than 56 per cent acetic acid and claimed the results obtained by this method were parallel to those obtained by Ost's method. They treated from one to two grams of the finely comminuted air-dry acetate (corrected for moisture content) with 20 to 30 cc. of water and 30 cc. of N sodium hydroxide for an hour at from 50° to 60° C., cooled and acidified the hydrolysate with 30 cc. of N hydrochloric acid, stirred the mixture thoroughly, and titrated it with N sodium hydroxide in the presence of phenolphthalein.

Fermazin (34) obtained checks within ± 0.2 per cent acetic acid when he saponified 5 grams of cellulose acetate with 50 cc. of 0.5 N potassium hydroxide 48 hours at room temperature, warmed the reaction mixture to 70° or 80° C., added an excess of 0.5 N sulfuric acid and titrated the excess of acid with 0.5 N potassium hydroxide.

Barnett (2) used Eberstadt's (31) preswelling of the acetate with acetone in a method of mild alkaline hydrolysis; he dissolved 0.3 gram of the ester in 30 cc. of acetone, added 47 cc. of 0.1 N sodium hydroxide and after 24 hours at room temperature diluted the hydrolysate and titrated the excess of alkali with 0.1 N acid in the presence of phenolphthalein. A blank determination with 0.3 gram of cellulose yielded volatile acids equivalent to 1.7 cc. of 0.1 N alkali. Bernoulli and his coworkers (10) obtained 54.88 per cent acetic acid by Barnett's method as compared with 54 ± 0.5 per cent by Ost's method. Murray, Staud, and Gray (95) obtained acetyl values of 39.6 and 39.4 when the ester was dissolved in 20 cc. of acetone and saponified with 20 cc. of 0.5 N sodium hydroxide at room temperature for 45 and 120 minutes respectively, and 39.8 for a treatment of 30 minutes at 53° C. Ridge, Parsons, and Corner (112) used N alkali to saponify samples of one gram of cellulose acetate rayon by Barnett's method.

Knoevenagel (77) used chloroform as a solvent for cellulose triacetate in an estimation of acetyl.

Battegay and Penche (6) employed 30 cc. of pyridine as a solvent for 0.3 to 0.5 gram of cellulose acetate, added 50 cc. of 0.5 N sodium hydroxide for 30 minutes at 25° C. and titrated the excess of alkali. Bernoulli and his coworkers (10) by this method obtained 54.6 per cent acetic acid as compared with 54 ± 0.5 per cent by Ost's method. Murray, Staud, and Gray (95) developed a method for the determination of the acetyl values of cellulose acetates of an acetyl content between 35 and 44 per cent; in this method 0.5 gram of the ester is dissolved in 20 cc. of pyridine in 15 minutes at 53° C. and saponified with 20 cc. of 0.5 N sodium hydroxide at 53° C. in 30 minutes. The excess of alkali is titrated with a standard acid in the presence of phenolphthalein. All their determinations checked within 0.6 per cent acetyl.

The disadvantages of the method of alkaline hydrolysis are that (a) the alkali acts on the cellulose acetate to produce acidic compounds other than acetic acid and that (b) any other acid present in the ester is estimated as acetic acid (65).

The distillation of the volatile acid after alkaline hydrolysis of the ester has been carried out by Cross, Bevan, and Beadle (21), Green and Perkin (43), Schwalbe (119), Ost and Katayama (100), and by Clément and Rivière (25). Cross, Bevan, and Beadle obtained 69.2 per cent acetic acid in this manner from an ester which gave a value of 73.1 by the acidimetric estimation of the hydrolysate. Green and Perkins added sulfuric acid in excess to the hydrolysate obtained by their method of alkaline hydrolysis, collected the distillate of ethyl acetate in alcoholic sodium

hydroxide, titrated the excess of alkali with a standard acid, and obtained values of 59.80 and 59.75 per cent acetic acid.

Schwalbe saponified 3 or 5 grams of cellulose acetate with 50 cc. of 25 per cent potassium hydroxide for 48 hours at room temperature, added 500 cc. of water, neutralized the mixture to Methyl Orange with concentrated sulfuric acid, and after 3 hours decanted the liquid from the precipitate, washed the residue in boiling water, acidified the combined filtrates with sulfuric acid, distilled these as in Ost's method, and obtained values of 58, 62.4, 60.7, and 62.9 per cent acetic acid.

Ost and Katayama saponified one gram of cellulose acetate with 20 cc. of 0.5 N alcoholic potassium hydroxide at room temperature for 24 hours, acidified the hydrolysate with sulfuric acid, added 5 grams of tartaric acid and distilled the mixture. The acid values obtained were corrected by blank determinations. Murray, Staud, and Gray used aqueous alkali as the saponifying agent, acidified the hydrolysate with phosphoric acid, added an excess of 30 per cent tartaric acid, collected 2 liters of distillate in standard alkali, titrated the excess of alkali with standard acid, and obtained acetyl values of 36.2 and 36.7 for one ester and of 43.9, 46.6, and 41.0 for another ester. Clément and Rivière employed 20 cc. of 95 per cent alcohol to swell one gram of cellulose acetate, added 20 cc. of N potassium hydroxide for saponification, neutralized the hydrolysate with N sulfuric acid, added 5 grams of tartaric acid and distilled the mixture with steam for four hours.

EXPERIMENTAL

The fibrous materials, white plain-woven fabrics of cellulose acetate rayon, cotton, degummed silk, or wool, and the chloroform-soluble cellulose triacetate of the Eastman Kodak Company, were prepared for analysis as in I.

The use of acetone as a differential solvent for cellulose acetate rayon

Approximately one gram of cellulose acetate rayon and one gram of cotton, silk, or wool were treated with 125 cc. of acetone for an hour; the mixture was then decanted through a 240-mesh sieve and the residue was rinsed by decantation with 5 portions of acetone of 20 cc. each. In order to insure complete rinsing at least 30 minutes were allowed for this process and the residue was thoroughly stirred in the acetone. The filtrate was diluted in an Erlenmeyer flask of a capacity of 500 cc. with 100 cc. of water and, in order to remove most of the acetone, was carefully (a capillary extending through the stopper to the bottom of the flask was effective in the prevention of bumping) distilled to 85° C. After the distillation 0.2 gram of sodium chloride was added to render the suspension more readily filterable and after 30 minutes the precipitated cellulose acetate was filtered into an alundum crucible and rinsed free from chloride. The crucible was placed in a weighing bottle and dried to constant weight. The results are given in table 1.

The gain in weight of the residual cellulose acetate was perhaps due to differences in humidity the days the initial and final weights were obtained as the ash content did not account for the increase in weight.

In eight experiments the cellulose acetate was precipitated from its

TABLE 1. *The estimation of cellulose acetate rayon by the acetone solution method*

Cellulose acetate rayon gram	Residual cellulose acetate		Fiber gram	Residual fiber percentage of union
	percentage of rayon	percentage of union		
1.0944	100.78	100.38	1.1312	100.29
1.0755	100.22	100.11	1.0577	99.98
1.1171	100.52	100.26	1.0801	100.33
1.0862	100.84	100.43		100.23
average	100.59	100.30		100.21
1.0839	100.01	100.00	1.0355	99.82
1.0632	100.52	100.27	0.9660	99.81
1.0776	100.63	100.32	1.0661	99.78
1.0890	—	—	0.9976	99.69
average	100.39	100.20		99.78
1.0725	100.08	100.04	0.9664	100.01
1.0765	99.89	99.94	0.9804	100.09
1.0848	100.22	100.12	0.9706	100.02
1.0836	100.47	100.24	1.0508	100.03
average	100.17	100.09		100.04

solution in acetone by the addition of 400 cc. of water and 0.5 gram of sodium chloride. After 5 days the precipitate was filtered, rinsed, and dried as described before. The results were inconsistent and ranged from a loss of 2.08 per cent to a gain of 2.04 per cent.

*The use of acetic acid as a differential solvent for
cellulose acetate rayon*

Approximately one gram of cellulose acetate rayon and one gram of cotton were treated with 125 cc. of 70 per cent acetic acid at 85° C. for 5 minutes; the mixture was then filtered through an alundum crucible and the residue was rinsed with five portions of 25 cc. each of 70 per cent acetic acid. In the determinations carried out in the absence of cotton, 125 cc. of 70 per cent acetic acid were added directly to the hot solution of the cellulose acetate and the ester was precipitated by the addition of 400 cc. of water and 0.5 gram of sodium chloride. After four days the mixture was filtered into an alundum crucible, rinsed with water, placed in a weighing bottle, and dried to constant weight. This method did not give dependable results; four determinations carried out in the absence of another fiber showed an average loss of 2.27 per cent of cellulose acetate and three analyses of mixtures of cellulose acetate rayon and cotton showed an average loss of 5.64 per cent of cellulose acetate.

Methods of acid hydrolysis

The determination of the acetyl value by the acid hydrolysis of cellulose acetate was carried out according to Ost (98) by treating approximately 3 grams of the ester with 10 cc. of 1:1 sulfuric acid for 48 hours, diluting the reaction mixture to 100 cc. with carbon-dioxide-free water and distilling it in a current of carbon-dioxide-free steam at the rate of 200 or 300 cc. of distillate per hour for four hours. The volume of the distillate was made up to a liter and portions of 100 cc. each were titrated

with 0.1 N sodium hydroxide in the presence of phenolphthalein (Methyl Red was used with protein fibers). The acetyl values obtained are given in table 2.

TABLE 2. *The estimation of the acetyl value by Ost's method*

Ester	gram	Volatile acid grams of acetic acid	Acetyl percentage
Cellulose acetate rayon	2.8893	1.5183	37.66
	3.1111	1.6351	37.67
	average		37.67
Cellulose triacetate	2.9169	1.7326	42.57
	2.9193	1.7416	42.76
	average		42.67

Blank determinations showed that samples of one gram of cotton, silk, or wool produced, respectively, the equivalent of 0.15, 0.12, and 13.35 cc. of 0.1 N acid and suggested that unions with cotton and silk if not with wool might be analyzed in this way. When mixtures were examined by this procedure approximately three grams each of cellulose acetate rayon and of cotton or silk were treated with 20 cc. of 1:1 sulfuric acid and the hydrolysate was diluted to 200 cc. The results are given in table 3.

TABLE 3. *The estimation of cellulose acetate by a modification of Ost's method*

Cellulose acetate rayon gram	Volatile acid grams of acetic acid	Acetyl percentage	Cellulose acetate rayon		Other fiber gram
			percentage of ester	percentage of union	
2.9111	1.5121	37.23	98.83		
2.8729	1.4656	36.56	97.05		
2.8780	1.4587	36.32	96.42		
average		36.70	97.43		
2.9065	1.4916	36.52	96.94	98.46	cotton 2.8822
2.9356	1.5053	36.52	96.95	98.38	2.6090
2.8588	1.4469	36.01	95.60	97.49	2.8907
average		36.35	96.50	98.11	
3.0662	1.5482	35.98	95.51	97.76	silk 3.0842
2.8722	1.4221	35.26	93.62	96.89	2.0169
average		35.62	94.57	97.33	

The method of acid hydrolysis has thus been shown unsatisfactory even for the analysis of mixtures of cotton or silk with cellulose acetate rayon because the dilution of the liquid to be distilled, in proportion to the weight of the sample and the concentration of the acid, gives too low acetyl values.

Methods of alkaline hydrolysis

The estimation of the acetyl value by the mildest method of alkaline hydrolysis was first carried out according to Barnett (2) by dissolving about 0.3 gram of cellulose acetate rayon in 30 cc. of acetone, adding 47 cc. of 0.1 N sodium hydroxide, and, after 24 hours, titrating the excess of

alkali with 0.1 N sulfuric acid in the presence of phenolphthalein. The results are given in table 4.

TABLE 4. *The estimation of the acetyl value by Barnett's method*

Cellulose acetate rayon gram	Acetic acid gram	Acetyl percentage
0.2800	0.1475	37.75
0.2731	0.1439	37.77
average		37.76

Blank determinations were made with cotton, silk, and wool (Methyl Red was the indicator used with the protein fibers) and are given in table 5.

Blank determinations by Barnett's method

	Fiber gram	Sodium hydroxide absorbed cc. 0.1 N
Cotton	1.1149	0.229
	1.0629	0.248
	1.0055	0.327
	1.0795	0.377
Silk	1.0348	2.211
	1.0065	2.250
	1.0577	2.940
	0.9802	2.972
Wool	1.0016	9.103
	0.9449	9.448
	0.9390	10.089
	1.0067	11.469

An attempt to develop a similar method using about 2 grams of cellulose acetate rayon, 150 cc. of acetone, 25 cc. of N sodium hydroxide, and 0.5 N sulfuric acid as the titrating solution failed because (a) the addition of the alkali reprecipitated the acetate as coarse lumps which absorbed large amounts of alkali and (b) the large volume of the acetone rendered the end-point very indistinct.

In another group of experiments alcohol was used as the swelling agent. About two grams of sample were immersed in 25 cc. of 95 per cent alcohol for 30 minutes. An equal volume of N sodium hydroxide was added and after 24 hours the excess of alkali was determined by titration with 0.5 N sulfuric acid. In four determinations the alcohol softened the cellulose acetate to such an extent that lumps formed which could not be broken up for titration. In three determinations the separation of the cellulose acetate fabric into yarns failed to prevent lumping. Since the solubility of cellulose acetate varies with the method of manufacture as well as with the acetyl content it is probable that the most effective concentration of alcohol must be determined for each ester. Blank determinations with cotton showed that samples of one, two, and three grams absorbed, respectively, the equivalent of 0.954, 1.110, and 1.111 cc. of 0.1 N sodium hydroxide.

The 1:1 alcohol-water solution used by Eberstadt (31) proved a very satisfactory swelling agent for the acetate rayon. Samples of about two

grams were immersed in 25 cc. of 1:1 alcohol-water solution for 15 hours before an equal volume of N sodium hydroxide was added. Then after 48 hours the reaction mixture was titrated as before. The acetyl values are given in table 6.

TABLE 6. *Acetyl values*

Ester	gram	Acetic acid gram	Acetyl percentage
Cellulose acetate rayon	1.9530	1.0260	37.65
	1.9856	1.0524	37.99
	2.0363	1.0831	38.12
	average		37.92
Cellulose triacetate	1.9436	1.2068	44.50
	1.9481	1.2113	44.56
	1.9486	1.2118	44.57
	average		44.54

Inconsistent results were obtained in the analysis of unions by this method when only 25 cc. of the swelling medium and of the alkali were used for a mixture of about 2 grams each of the acetate rayon and cotton. The results ranged from a loss of 19.58 per cent to a gain of 0.13 per cent of the acetate rayon.

The procedure was modified for the analysis of acetate rayon and cotton unions by the use of double the volume of the swelling medium and the alkali. On the basis of blank determinations a correction of 0.0117 grams of acetate rayon was made in the experiments reported in table 7.

TABLE 7.

Cellulose acetate rayon	Acetic acid	Acetyl	Cellulose acetate rayon (calculated)		Cotton
			percentage of ester	percentage of union	
gram	gram	percentage			gram
1.6298	0.8798	38.69	101.31	100.59	1.9768
1.6536	0.8963	38.84	101.72	100.75	2.1398

SUMMARY

1. The use of acetone as a differential solvent for the cellulose acetates (other than the triacetate) of union textiles gives the best results and is the only method which may be used with all union textiles. The results obtained by the method of direct solution are about the same (mean error of +0.20 per cent) as those based on the weight of the residue; however, the indirect method is preferable because it is more rapid and is simpler of manipulation.

2. Acetic acid has been shown unsatisfactory as a differential solvent in the direct estimation of the cellulose acetate of union textiles.

3. The proximate analysis of a union textile for cellulose acetate by means of the determination of the acetyl value by the method of acid hydrolysis has been shown unsatisfactory.

4. The use of 1:1 alcohol-water as a swelling medium and 0.5 N

alcoholic sodium hydroxide as a saponifying agent has been shown the best (mean error of +1.52 per cent ester) of all the methods of alkaline hydrolysis for the acetyl value of cellulose acetate rayon in cotton mixtures.

5. The cellulose acetate rayon studied (of usual acetyl content as compared with 15, 18, 24, 26, 47, 84, 97, 105 and 126) yielded acetyl values of 37.67, 37.76, and 37.92 (the theoretical acetyl value for cellulose diacetate is 34.96), respectively, by Ost's, Barnett's, and Eberstadt's methods.

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PLATE I

Extraction of Textiles with Ether

PLATE I



PHYSIOLOGICAL STUDIES OF THE BUTYL-ACETONE GROUP OF BACTERIA

I. GELATINOLYSIS¹

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Results are reported on the gelatinolytic properties of twenty-two cultures of the butyl-acetone group of bacteria. The ability of these bacteria to bring about gelatinolysis is of importance to the bacteriologist in the identification of species and is of some practical significance; for example, the Weizmann patent under which the commercial production of butyl alcohol and acetone is licensed, specifies that the organism used is characterized by its power of gelatinolysis. It seemed desirable to put the whole matter on a firm basis by making comparative studies by improved methods of the more important strains and species available.

METHODS

The procedures chosen for the determination of gelatinolysis may be referred to as follows: (a) method of maximum temperature of gelation, (b) mercury-protein precipitation method and (c) method of determination of viscosity. Each of the three will be discussed separately.

Maximum temperature of gelation. The purpose of the method is, as indicated by the title, to detect the maximum temperature at which the gelatin culture solidifies after incubation at 37° C. The method is applicable especially to those organisms with an optimum growth temperature above the gelation point of the medium. In the work here presented the gelation temperature was 20.5° C.

The medium employed consisted of gelatin (Difco) 10 per cent, peptone 0.5 per cent, glucose 0.4 per cent, dipotassium phosphate 0.1 per cent and was sterilized for 15 minutes at 20 pounds pressure in the autoclave. After removal from the autoclave, the medium was cooled to 37° C. by plunging into cold water. The medium was inoculated when 37° C. was reached.

The organism was cultured serially in the medium at 37° C. and the third transfer after incubation for 24 hours (or longer) was placed in the water bath at approximately 37° C. and the temperature slowly lowered, especially as 20.5° C. was approached, as this is the temperature at which the controls were found to solidify. A drop of one degree required 10 minutes on an average. The tubes were periodically inclined to the horizontal position and the gelation temperature noted as that point at which the medium *failed to break* from the wall of the horizontal tube. Slight deformation of the surface took place but *no flow* occurred.

Control uninoculated tubes of the medium gave constant and reproducible results within 0.5° C., varying from 20 to 20.5° C. Non-liquefying forms gave substantially the same temperature of gelation as the controls

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and did not vary significantly during the period of incubation. The only precaution against evaporation was the use of tightly fitting cotton plugs; this precaution is not essential since the controls are subject to the same conditions as the inoculated tubes and a comparison is made.

Mercury-protein precipitation method. The procedure employed is an adaptation of the method described by Frazier (1926) to anaerobic, aerogenic cultures of the butyl type. For details of the principles underlying the test, reference is made to the work of Frazier.

The medium employed was composed of: gelatin (Difco) 0.4 per cent, glucose 0.4 per cent, peptone 0.5 per cent, dipotassium phosphate 0.1 per cent, agar 2.0 per cent. Long slants are prepared and approximately five cubic centimeters of inoculum are placed in each tube. In this way the upper half of the slant which is not covered by inoculum may serve as a control as the tube is incubated in a vertical position. The inoculum should be taken from a young culture just starting to produce gas. Such a culture medium for inoculum should be preferably of the same composition as the agar gelatin medium employed, with the agar, of course, omitted. However, the final results were not different when corn-mash or glucose-peptone media were employed. The slants are incubated at 37° C. for 48 to 72 hours when they are removed and prepared for the addition of the acid bichloride of mercury solution. The liquid culture medium is poured from each tube and the agar-gelatin slant removed by flooding it out with water. The surface of the slant is carefully washed in running water by means of a cotton swab on the end of an applicator or match to remove adhering bacterial growth which interferes with the test. The washed slant is then placed in a bath composed of mercuric chloride 15 grams, concentrated hydrochloric acid 20 cc. and 100 cc. of water. Gelatinolysis is indicated within a few minutes by a clear medium whereas, the presence of unattacked gelatin (upper portion of the slant) is indicated by a chalk-like whiteness of the medium. This method yielded excellent results. The technic is simple and provides for the growth of aerogenic anaerobes. Since an active culture is used in making the inoculation we have found that the reduced potential necessary for the growth of anaerobes of the butyl-acetone type is adequately maintained during transfer of the inoculum to the slant. Redox potential determinations made by means of a vacuum-tube potentiometric set-up gave Eh values as follows: original culture -0.286; immediately after transfer of inoculum to slant, -0.270; five minutes after transfer, -0.280.

We have tried a number of protein precipitating agents such as phosphotungstic and trichloroacetic acids, in the effort to find the most satisfactory. Tannic acid has been used and a word regarding the results should

TABLE 1. *Precipitating action of tannic acid and mercuric chloride*

	Gelatin	Protease-peptone	Peptone	Aminoids (casein)	Amino acids: glycine, tyrosine or phenylalanine
Acid HgCl ₂	Heavy ppt.	Light ppt.	Trace	No. ppt.	No ppt.
Tannic acid	Heavy ppt.	Heavy ppt.	Moderately heavy ppt.	Light ppt.	No ppt.

be mentioned. Tannic acid is not suitable because of its property of forming a precipitate with degradation products of proteins. Solutions were prepared of gelatin, proteose-peptone (Difco) peptone (Difco) casein aminoids (Arlington) and three amino acids (glycine, phenylalanine and tyrosine). The acid bichloride and tannic acid solutions were added to each of the solutions and the precipitation noted.

It is apparent from the results in table 1 that unless the organisms break down the gelatin and any other proteins in the medium to the amino acid stage (polypeptid?), the reaction with tannic acid will not show any degradation of the protein unless the precipitate can be qualitatively distinguished in some manner. Apparently we met with such a situation with the butyl organisms for although the gelatin was certainly attacked, it was not broken down apparently into amino acids, at least completely, for a culture of 2C which is a good liquefier, was incubated for one week at 37° C. and following good growth, was then tested by the addition of tannic acid solution as recommended by Frazier (1926). The result was an opaque, white precipitate which would indicate proteins or their hydrolytic products above the amino acids.

Viscosity method. Torrey (1910) used the Ostwald viscometer at 40° C. to determine the viscosity of gelatin cultures which had been incubated at 36° C. and then placed at 50° C., before making the viscosity determinations. The immediate object of Torrey's test was to aid in the identification of *Escherichia coli* in routine water analysis. Precautions pointed out by the author are: (a) necessity of having an exact quantity of medium in the Ostwald viscometer, (b) control of evaporation and (c) use of same lot of gelatin. He also pointed out that cultures forming a slime may not prove suitable for this method.

Levine and Carpenter (1923), Levine and Shaw (1924) and Shaw (1924) also recommended the use of the Ostwald viscometer. The latter authors used a two per cent gelatin medium and following the suggestion of Davis and Oakes (1922) preheated the medium at 50° C. (rejuvenation) for 20 minutes before making the viscosity determinations at 40° C. These authors showed that liquefaction could be detected before any change in formal titration took place.

The determinations of viscosity in the present work in one series of experiments were made at 40° C. following rejuvenation at 50° C. for 20 minutes. The medium employed was composed of gelatin 2.0 per cent, glucose 0.4 per cent, dipotassium phosphate 0.1 per cent, peptone 1.0 per cent. The cultures were serially transferred at least three times in this medium. Incubation took place at 37° C. The apparatus for the determination of viscosity consists of a museum jar fitted with a thermostatically controlled, electric immersion heater, stirring motor and stirrer, wire basket to provide storage for tubes attaining correct temperature (40° C.), one or two tested thermometers, one placed with mercury near viscometer storage bulb, the other thermometer placed at some distance to check uniformity of temperature in the bath, and finally one or two Ostwald viscometers suspended in the water so that the gelatin is below the water level at all times. A stop-watch is essential in taking the time of flow between the two marks on the viscometer. After a predetermined period of incubation at 37° C., the tube of gelatin medium is heated for 20 minutes at 50° C. (rejuvenation); it is then centrifuged to precipitate any debris. A definite quantity is placed in the viscometer by means of a pipette and

the apparatus placed in the water bath at 40° C. and time allowed for the gelatin to attain temperature. The cultures may be placed in a basket in the bath and allowed to attain temperature. The upper bulb is then filled to the mark and the exact time required to empty is noted. Duplicate readings should check within a fraction of a second usually varying not more than 0.2 second.

It is more convenient to use a *modified viscometer* especially when it is desired to plot determinations of viscosity against time of incubation. This apparatus provides an economical and simple culture tube and viscometer combined. Furthermore it is adapted to the cultivation of vigorously aerogenic forms whereas, the use of the Ostwald viscometer is impracticable due to the great number of bubbles which collect on top of



Fig. 1.
Modified
viscometer.

the medium and sediment which prevent taking of accurate readings. A sketch of this modified apparatus is shown in figure 1. It consists of a straight piece of capillary tubing to which a bulb is sealed with a short extension of tubing. The capillary tube is inserted through a rubber stopper which fits an ordinary 15 cm. test tube. An additional glass tube is provided in the stopper to permit escape of gas and to allow flow of air when the medium is forced into the viscometer bulb prior to the test. The upper end is flared and loosely filled with cotton to prevent contamination. Should the cotton plug fit too lightly the time of discharge of the viscometer will be changed. Inoculation of the medium may be made by means of a pipette through this tube. A similar plug is placed in the upper portion of the viscometer. The use of a cotton plug has caused no difficulty. It is important to insert the capillary into the medium to a fixed depth; this should be sufficient to fill easily the viscometer bulb with medium. An etched ring is placed on the capillary to mark the position and should be placed at approximately the same distance from the end of all viscometers. The capillary used has been approximately of one millimeter bore and the bulb had a capacity of about three cubic centimeters. The apparatus may be constructed easily in the laboratory.

The medium is placed in the test tube and the rubber stopper with the plugged viscometer and outlet tube is fixed in the test tube and the whole sterilized in the autoclave at 15 pounds for 20 minutes. Gauze may be wrapped around the tube and stopper with the latter fitting loosely the tube. The inoculation is made when the medium has cooled and the apparatus suspended in the water-bath at 37° C. It was not found necessary to rejuvenate the gelatin within the time limit of our experiments with incubation at 37° C. The control uninoculated tubes showed no increase of viscosity for a period of two days which was ample to complete the experiment. Furthermore, after completing the viscosity determinations at 37° C. over the period of the experiment (48 hours) we have rejuvenated and redetermined viscosity and have found that rejuvenation did not alter the readings.

In each case the viscosity determinations made by means of the culture tube viscometer are expressed as a ratio

$$\frac{\text{Time of outflow of medium after inoculation}}{\text{Time of outflow of medium before inoculation}}$$

If there is no marked change of viscosity with time the ratio remains approximately one. An increase in viscosity is manifested by a ratio greater than one, a decrease by a ratio less than one. Inasmuch as the quantity of inoculum used is frequently as much as one twentieth of the volume of the medium inoculated, there is often a shift in the ratio immediately following inoculation. This viscosity ratio when plotted against time shows graphically the relative change in viscosity. A control tube is used to determine whether there is any departure from the viscosity ratio of 1.0 during the progress of the test. If a marked increase in viscosity had been shown by the check, it would have been necessary to rejuvenate the gelatin by heating.

The culture-tube viscometer has proven very satisfactory in our hands. It is economical, permits repeated readings, avoids the necessity of centrifuging or filtering and transferring of the medium to a viscometer, is applicable to aerogenic bacteria, and does not require the use of an exact amount of medium. The latter point is a decided advantage.

The culture-tube viscometer provides a convenient, rapid and quantitative method for the determination of gelatinolysis.

Again it should be noted that rejuvenation of the gelatin may be neces-

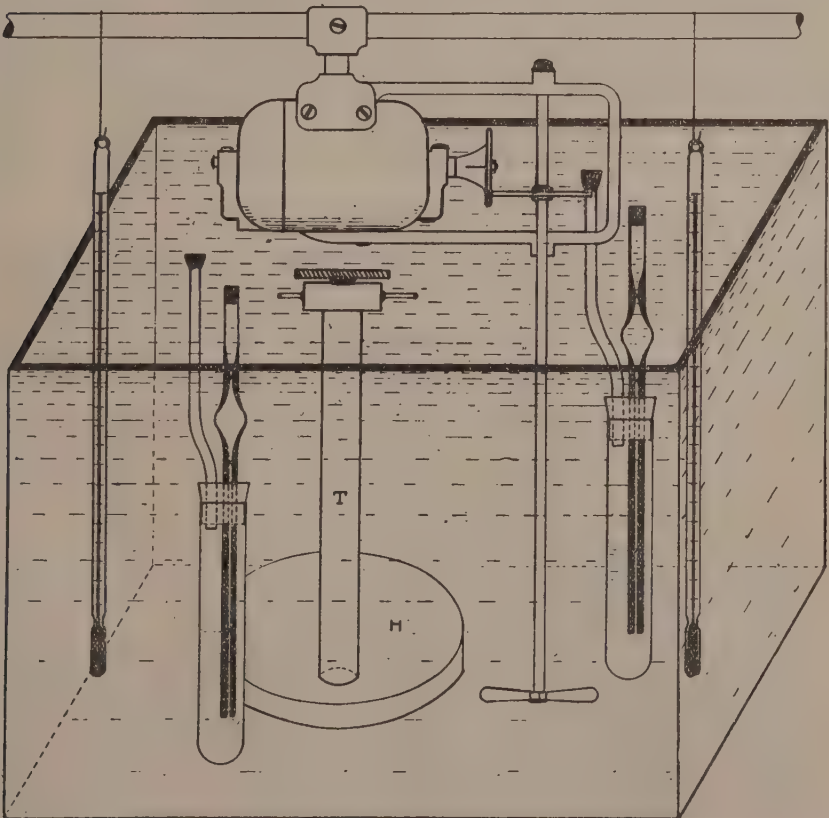


Fig. 1A. Apparatus for use of modified viscometer.

sary if the control tube shows departure from a viscosity ratio of one. However, under the conditions of our experiments rejuvenation was not necessary.

The complete apparatus and set-up is shown in figure 1A.

In the three media employed it will be noted that glucose (0.4 per cent) was used. This concentration was decided upon after trial. In the absence of any utilizable carbohydrate the butyl-acetone bacteria fail to grow. Growth in a gelatin-peptone medium was not sufficient to give satisfactory results although the presence of gelatinase could be detected. The objection which might arise to the use of glucose, especially in greater concentration than used in this work, lies in the fact that its presence might inhibit the production of gelatinase, a fact in the case of certain bacteria such as *Proteus*. Only after complete utilization of the sugar is the enzyme produced. The use of 0.4 per cent dextrose did not prevent the formation of gelatinase since it was completely dissimilated by the bacteria. It will be noted in the graphs to be presented that liquefaction became apparent in liquefying cultures following the period of active gas production.

The effect of change in pH of the culture on viscosity does not in any way, tend to reduce the usefulness of the viscosity method of determining gelatinolysis. The work of Davis and Oakes (1922) shows the maximum viscosity to be at pH 3.5 (40° C.) with a diminution on either side of this acidity to a minimum at pH 8.0. Our cultures had a final pH very near to 4.7. Thus there would be a slight increase of viscosity to be ascribed to a change in pH since our culture medium had an initial pH of 6.8 to 7.0. For practical purposes the influence of pH may be disregarded in the present work since the effect is slight and results in an increase of viscosity.

CULTURES

The species and strains used in the present experimental work retain the names by which they were received. The source of each culture is indicated as follows:

Organism received as	Culture number	Received from
<i>Clostridium acetobutylicum</i> —B	1C	Dr. Leo F. Rettger, Yale University
" " —I	2C	"
" " —K	3C	"
" " —M	4C	"
" " —R	5C	"
" " —So	6C	"
" " —St	7C	"
" "	14C	Dr. A. M. Wynne, University of Toronto
" "	1D	Dr. A. J. Kluyver, Delft, Holland

<i>Cl. acetonigenum</i> —862	13C	American Type Culture Collection
<i>Cl. Beijerinckii</i> —858	9C	"
" "	4D	Dr. A. J. Kluyver, Delft, Holland
<i>Cl. butylicum</i>	2D	"
<i>Bacillus butylicus</i> —3625	15C	American Type Culture Collection
" " —4259	16C	"
<i>Cl. butyricum</i> —824	8C	"
<i>Cl. felsineum</i>	5D	Dr. A. J. Kluyver, Delft, Holland
<i>Cl. Pasteurianum</i> —861	12C	American Type Culture Collection
" "	6D	Dr. A. J. Kluyver, Delft, Holland
<i>Clostridium pectinovorum</i> —859	10C	American Type Culture Collection
<i>Cl. saccharobutyricum</i> —860	11C	"
" "	3D	Dr. A. J. Kluyver, Delft, Holland

Appreciation is expressed to the various donors for their kindness in sending cultures.

TABLE 2. *Maximum temperatures of gelation*

Organism received as	Culture no.	Maximum gelation temperature, C°		
		24 hrs.	48 hrs.	72 hrs.
Control		20.5	20.5	20.5
<i>Clostridium acetobutylicum</i>	1C	20.0	16.5	10.5
" "	2C	15.0	2.5	—5.0 (liquid)
" "	3C	16.0	—4.0	
" "	4C	20.5	19.5	17.0
" "	5C	17.0	2.5	—5.0 (liquid)
" "	6C	20.5	17.0	
" "	7C	14.0	—4.0	
" "	14C	16.5	2.0	—5.0 (liquid)
" "	1D	11.5	3.0	
<i>Cl. acetonigenum</i>	13C	20.5	18.5	16.5
<i>Cl. Beijerinckii</i>	9C	20.5	20.5	20.5
" "	4D	20.5	20.5	20.5
<i>Cl. butylicum</i>	2D	20.5	20.5	20.5
<i>Bacillus butylicus</i>	15C	13.0	1.5	
" "	16C	20.0	19.5	17.0
<i>Cl. butyricum</i>	8C	15.5	2.0	—5.0 (liquid)
<i>Cl. felsineum</i>	5D	16.0	1.0	
<i>Cl. Pasteurianum</i>	6D	20.5	20.5	20.5
" "	12C	20.5	20.5	20.5
<i>Cl. pectinovorum</i>	10C	20.5	20.5	20.5
<i>Cl. saccharobutyricum</i>	11C	20.5	20.5	20.5
" "	3D	20.5	20.5	20.5

EXPERIMENTAL RESULTS

The experimental results obtained by each method will be presented separately and discussed in the summary.

Maximum temperature of gelation. These results are presented in table 2. Each non-liquefying culture is characterized by a constant gelation temperature (20.5° C.) throughout the period of the experiment (72 hrs.).

The various strains and species which did not liquefy gelatin showed exactly the same maximum temperature of gelation (20.5° C.) at intervals of 24, 48 and 72 hours. It may be concluded that non-liquefying cultures do not change the maximum temperature of gelation during three days' growth at 37° C.

Of 22 strains, 14 are to be classed as liquefiers. The liquefiers do not show the same consistency as the non-liquefiers. This may be due, in part, to inherent defects of the method.

However, remarkable consistency and uniformity of results are hardly to be expected when we consider the difficulty of obtaining cultures even of the same organism, in exactly the same stage of development at any fixed time.

A number of cultures were sufficiently gelatinolytic as to destroy apparently all the gelation properties of the medium within 48 hours [cultures 2C, 3C, 5C, 7C, 14C, 1D (*Cl. acetobutylicum*), 8C (*Cl. butyricum*) and 5D (*Cl. felsineum*)]. In a number of instances the medium was cooled to -5° C. and no gelation of the medium occurred. In these cases a crystal of ice dropped into the medium at the above temperature caused prompt solidification, showing a "super-cooled" condition.

In cultures 1C, 4C, 6C, (*Cl. acetobutylicum*), 13C (*Cl. acetoniigenum*) and 16C (*B. butylicus*) the liquefying properties were not so pronounced as in those just discussed. It is not apparent whether this reduced liquefying power is a matter of previous history of the culture or not. Frequently when such cultures were tested again greater liquefaction resulted, indicating that the apparent difference lay in the culture itself. Every effort was made to have the cultures in an active state and in the same stage of development. *Bacillus butylicus*, 16C in the experiment recorded in table 2 did not appear from the results to be as vigorous a liquefier as culture 15C which was received under the same name. However, a later test showed 16C to liquefy nearly as well as 15C (14° C. for 16C and 10° C. for 15C at 48 hours). Culture 6C likewise showed more marked liquefaction in another test (0° C., 48 hours) than in the experiment recorded in table 2. These differences in results were the only ones noted and are of a quantitative nature.

Mercury-protein precipitation method. The results obtained by the use of this method are shown in table 3 and photographs shown in plate I. The method proved very convenient and yielded excellent results when used as described.

The results confirm those of the previous method in establishing the identity of the liquefying and non-liquefying strains. The photograph in plate I was taken with a black background which is visible through the clear medium of the liquefiers (B and D). Slant A was prepared by immersing the uninoculated medium in the acid mercuric chloride bath for several minutes, while C, a non-liquefier, was prepared in the same manner as B and D by first washing off the organisms and then placing in the bath.

TABLE 3. Gelatinolysis by the mercury-protein precipitation method

Organism received as	Culture no.	Effect of HgCl ₂ on 72 hour cultures	Gelat-inolysis
Control		Opaque white	
<i>Clostridium acetobutylicum</i>	1C	Clear	+
" "	2C	Clear	++
" "	3C	Clear	++
" "	4C	Clear	++
" "	5C	Clear	++
" "	6C	Clear	++
" "	7C	Clear	++
" "	14C	Clear	++
" "	1D	Clear	++
<i>Cl. acetonigenum</i>	13C	Clear	++
<i>Cl. Beijerinckii</i>	9C	Opaque white	—
" "	4D	Opaque white	—
<i>Cl. butylicum</i>	2D	Opaque white	—
<i>Bacillus butylicus</i>	15C	Clear	++
" "	16C	Clear	++
<i>Cl. butyricum</i>	8C	Clear	++
<i>Cl. felsineum</i>	5D	Clear	++
<i>Cl. Pasteurianum</i>	12C	Opaque white	—
" "	6D	Opaque white	—
<i>Cl. pectinovorum</i>	10C	Opaque white	—
<i>Cl. saccharobutyricum</i>	11C	Opaque white	—
" "	3D	Opaque white	—

The chalk-like whiteness is apparent. The photograph was made from unselected tubes used in the experiments and clearly differentiates the liquefiers and non-liquefiers.

Viscosity determinations. Viscosities determined by the Ostwald viscometer are given in table 4, as ratios. These results are in agreement with

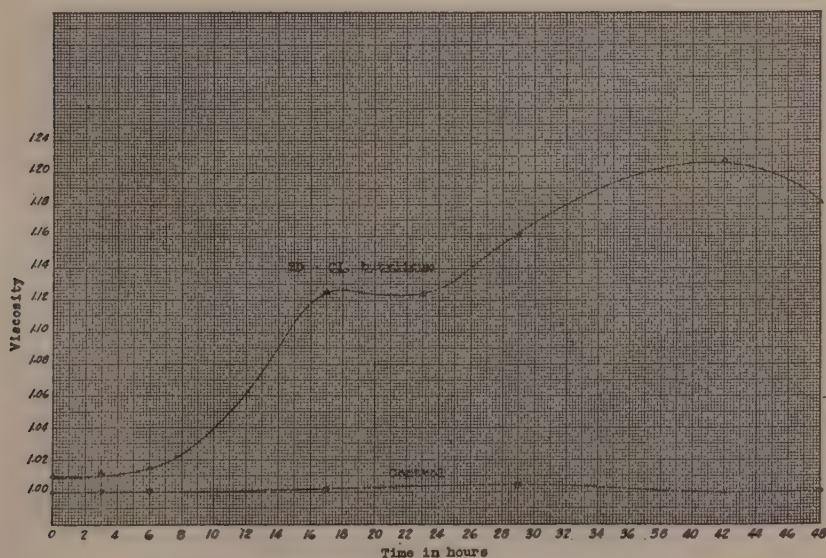
Fig. 2. Viscosity graph of *C. butylicum*, a slime former.

TABLE 4. Viscosity* as determined by the Ostwald viscometer

Organism received as	Culture no.	Age of culture					
		4 hrs.	24 hrs.	48 hrs.	72 hrs.	120 hrs.	14 days
Control		1.000	1.006	1.000	1.020	1.000	
<i>Clostridium acetobutylicum</i>	1C	0.974	0.832	0.814	0.807	0.774	
" "	2C	0.967	0.929	0.787	0.767		0.767
" "	3C	0.967	0.877	0.781	0.781		
" "	4C	0.967	0.852	0.807	0.807		
" "	5C	0.967	0.820	0.800	0.794		
" "	6C	0.974	0.956	0.877	0.864		
" "	7C	0.955	0.824	0.800		0.774	
" "	14C	0.948	0.807		0.800	0.774	
" "	1D	0.980	0.820	0.820	0.800		
<i>Cl. acetonigenum</i>	13C	1.006	0.935	0.910	0.864	0.832	
<i>Cl. Beijerinckii</i>	9C	1.006	1.000	1.000	1.006		
" "	4D	1.013	1.014	0.998	0.967	0.960	
<i>Cl. butylicum</i>	2D	1.013	1.038	0.994	1.031		1.000
<i>Bacillus butylicus</i>	15C	0.974	0.903	0.814	0.774		0.760
" "	16C	0.942	0.858	0.814	0.781		
<i>Cl. butyricum</i>	8C	0.967	0.826	0.775	0.767		0.767
<i>Cl. felsineum</i>	5D	0.967	0.823	0.790			
<i>Cl. Pasteurianum</i>	12C	1.006	0.994	0.988	1.006		
" "	6D	1.006	0.980	1.000	0.956	0.950	1.000
<i>Cl. pectinovorum</i>	10C	1.011	0.988	0.988			
<i>Cl. saccharobutyricum</i>	11C	1.006	1.000	0.980	0.980		
" "	3D	1.010	0.998	0.990	0.988		

* Viscosity recorded as: $\frac{\text{discharge time of culture}}{\text{discharge time of uninoculated medium}}$

those obtained by the former methods and definitely show *Cl. acetobutylicum*, *Cl. felsineum*, *Cl. butyricum*, *Cl. acetonigenum* and *B. butylicus* to be gelatin liquefiers. *Cl. Beijerinckii*, *Cl. pectinovorum*, *Cl. saccharobuty-*

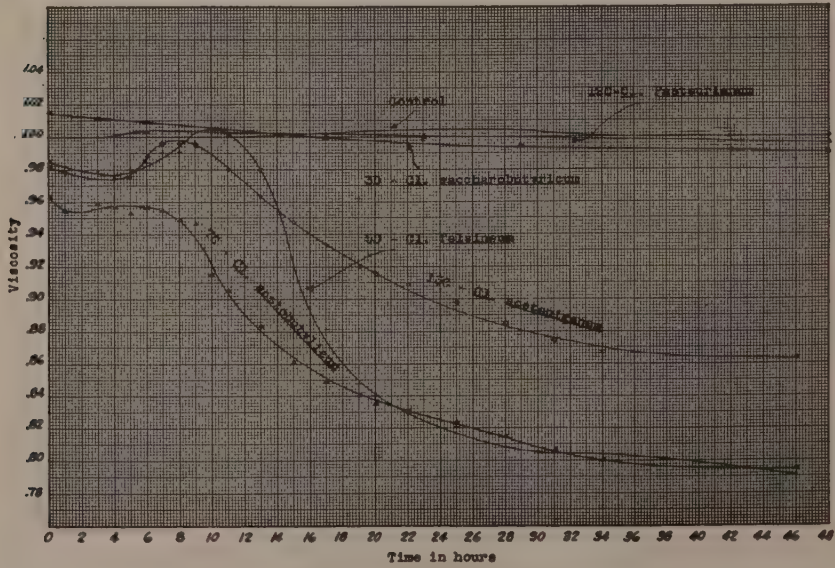


Fig. 3. Viscosity graphs.

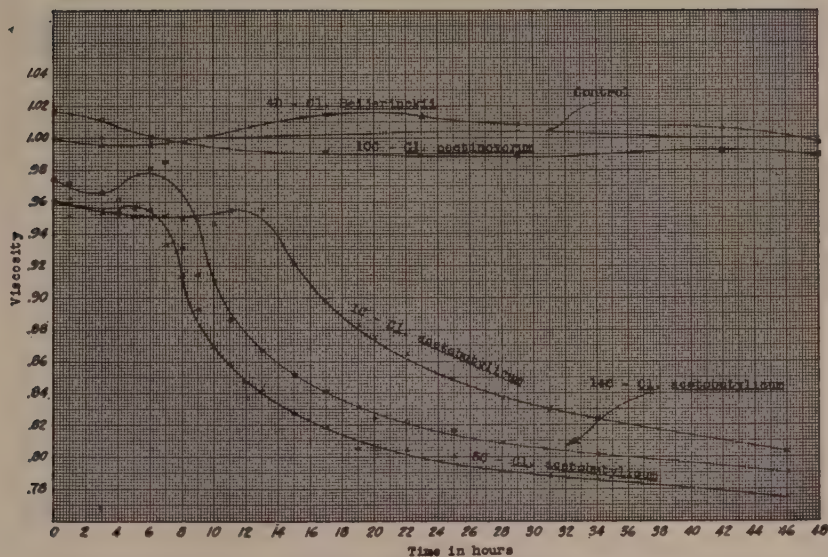


Fig. 4. Viscosity graphs.

ricum, *Cl. Pasteurianum* and *Cl. butylicum* are non-liquefiers. Observation of the results after 72 hours (table 4) reveals the fact that no liquefier showed a viscosity ratio above 0.864 (strain 6C) while the lowest viscosity shown by a non-liquefier was that of *Cl. Pasteurianum* giving a reading of 0.956. It is to be recalled that each determination of viscosity by the Ostwald viscometer is made on an individual tube of a series inoculated at the start of the experiment. Use of the Ostwald viscometer as a culture tube is impracticable with aerogenic organisms of the butyl-acetone group. Inasmuch as it is difficult or practically impossible to obtain a series of six cultures of an organism in exactly the same stage of growth at any given time, it becomes apparent that plotting of viscosity with time may lead to irregularities in the graph. For this reason the graphs shown in figures 2, 3 and 4 are based on the results obtained by the use of the culture-tube viscometer. These data are tabulated in tables 5, 6, 7 and 8. Here again the results confirm previous data with regard to the ability of strains and species to liquefy gelatin.

Cl. butylicum strain 2D is a slime producing organism whose viscosity graph is shown in figure 2. The organism is not a liquefier of gelatin. Apparently the slime causes an increase in viscosity resulting in a viscosity ratio greater than the 1.0 of the control. Failure of table 4 to show any marked increase of viscosity for 2D is due to centrifugalization of the slime. It is possible that slime production would interfere with determination of gelatinolysis by viscosity measurements. Further experimental data are required to answer this question. In view of the simplicity of the methods, it seems desirable in describing species of bacteria, to determine gelatinolysis by at least two different methods.

A slight rise in the curves of several strains occurred at the time of maximum gas production and seemed to be limited to the liquefying cul-

TABLE 6. (Continued) Viscosity determined by the culture tube viscometer
Liqueliers.

Organism received as	Culture No.	Discharge time (seconds)		Discharge time (seconds) after incubation at 37° C. (hours)											
		Uninoculated gelatin medium	Water												
				13	15	17	19	20	22	25	28	31	34	46	
Control		47.9	30.7	47.9	47.9	47.8	47.7	47.8	47.7	47.7	47.8	47.5	47.7	47.6	
<i>Clostridium acetobutylicum</i>	1C	94.8	60.8	90.5	87.4	85.1	83.7	83.0	82.0	80.5	79.4	78.7	78.1	76.2	
"	3C	54.9	35.2	46.7	45.8	45.0	44.5	44.3	44.2	43.8	43.6	43.2	43.0	42.3	
"	5C	81.0	51.9	68.2	67.0	66.3	65.2	65.2	65.1	64.8	64.1	63.8	63.6	62.7	
"	7C	52.1	33.4	46.0	44.9	44.3	43.8	43.5	43.2	42.9	42.4	42.0	41.7	41.4	
"	14C	53.4	34.2	46.3	45.5	44.9	44.4	44.0	43.9	43.6	43.2	42.8	42.8	42.2	
<i>C. acetogenicum</i>	13C	77.1	49.4	74.3	73.1	72.0	71.0	70.6	70.1	69.2	68.2	67.4	66.9	65.5	
<i>C. felsineum</i>	5D	55.7	35.7	54.6	51.3	49.1	47.2	46.8	46.2	45.7	45.0	44.8	44.8	44.0	

TABLE 7. Viscosity determined by the culture tube viscometer
Non liqueliers.

Organism received as	Culture no.	Viscosity ratio after incubation at 37° C. (hours)											
		0	3	6	17	23	29	42	48				
Control		1.000	1.000	1.000	1.002	1.000	1.004	1.000	1.000				
<i>Clostridium Beijerinckii</i>	9C	1.006	1.004	1.005	1.027	1.023	1.021	1.017	1.019				
"	4D	1.000	0.996	0.996	1.016	1.014	1.008	1.006	0.998				
<i>C. butylicum</i>	2D	1.010	1.011	1.015	1.125	1.124	1.161	1.207	1.180				
<i>C. Pasteurianum</i>	12C	1.000	1.000	1.013	1.001	1.000	1.000	0.997	0.996				
"	6D	1.002	1.002	0.996	0.994	0.996	0.996	0.996	0.990				
<i>C. pectinovorum</i>	10C	1.016	1.011	1.001	0.991	0.989	0.988	0.992	0.989				
<i>C. saccharobutylicum</i>	11C	0.994	0.992	0.996	0.988	0.984	0.984	0.982	0.980				
"	3D	1.015	1.011	1.009	1.000	0.998	0.994	0.992	0.990				

Liquefiers.
TABLE 8. *Viscosity determined by the culture-tube viscometer*

Organism received as	Culture no.	Viscosity ratio after incubation at 37° C. (hours)										
		0	1	3	4	5	6	7	8	9	10	11
Control	10	1.000	1.000	1.006	1.006	0.998	0.996	1.000	1.002	0.996	0.998	0.996
<i>Clostridium acetobutylicum</i>	3C	0.960	0.951	0.954	0.951	0.951	0.950	0.951	0.949	0.949	0.946	0.954
"	5C	0.967	0.961	0.951	0.954	0.961	0.967	0.965	0.940	0.917	0.883	0.871
"	7C	0.963	0.962	0.953	0.954	0.956	0.955	0.932	0.913	0.892	0.869	0.859
"	14C	0.974	0.954	0.959	0.956	0.952	0.956	0.954	0.948	0.946	0.915	0.905
<i>Cl. acetogenicum</i>	13C	0.982	0.972	0.966	0.961	0.968	0.981	0.985	0.931	0.914	0.901	0.886
<i>Cl. felsineum</i>	5D	0.986	0.982	0.979	0.977	0.986	0.988	0.996	0.996	0.995	0.981	0.981
						0.980	0.984	0.991	0.991	0.996	1.005	1.002

TABLE 8. (Continued) *Viscosity determined by the culture-tube viscometer*
Liquefiers.

Organism received as		Culture no.	Viscosity ratio after incubation at 37° C. (hours)											
			13	15	17	19	20	22	25	28	31	34	46	
Control		10	1.000	1.000	0.998	0.996	0.998	0.996	0.996	0.998	0.992	0.996	0.994	
<i>Clostridium acetobutylicum</i>		30	0.955	0.922	0.898	0.883	0.875	0.865	0.849	0.838	0.830	0.824	0.804	
"		50	0.851	0.834	0.819	0.810	0.807	0.805	0.798	0.794	0.787	0.783	0.771	
"		70	0.842	0.827	0.818	0.805	0.805	0.804	0.800	0.791	0.788	0.786	0.775	
"		140	0.883	0.861	0.849	0.840	0.835	0.829	0.823	0.814	0.806	0.800	0.794	
"		130	0.867	0.852	0.841	0.832	0.824	0.822	0.817	0.809	0.809	0.802	0.790	
<i>Cl. acetogenicum</i>		50	0.964	0.948	0.934	0.921	0.916	0.909	0.898	0.885	0.874	0.868	0.863	
<i>Cl. felsineum</i>			0.980	0.921	0.882	0.847	0.840	0.829	0.821	0.808	0.804	0.804	0.790	

tures. The cause was not determined but may be a manifestation of the tendency of the butyl-acetone group to form slime.

By use of the culture-tube viscometer, smooth curves showing the progress of liquefaction are obtained with little difficulty, leading to a distinct separation of the liquefiers from non-liquefying strains.

From the standpoint of interpretation of the results in tables 7 and 8, it is apparent that the non-liquefiers taken at 48 hours did not yield viscosity ratios of less than 0.980 (*Cl. Pasteurianum*, 6D). On the other hand no liquefier gave a value greater than 0.863 (*Cl. acetonigenum*, 13C). It is doubtful whether there is any advantage in continuing the determinations after 48 hours in the case of the present work.

SUMMARY AND CONCLUSIONS

Determination of gelatinolysis of 22 species and strains of the butyl-acetone group of bacteria by three different and improved methods has led to the conclusion that the following are to be classed as gelatin liquefiers:

1. *Cl. acetobutylicum*, 1C, 2C, 3C, 4C, 5C, 6C, 7C, 14C, 1D
2. *Cl. felsineum*, 5D
3. *Cl. butyricum*, 8C
4. *Cl. acetonigenum*, 13C
5. *B. butylicus*, 15C, 16C

The following are not gelatin liquefiers:

1. *Cl. butylicum*, 2D
2. *Cl. Pasteurianum*, 6D, 12C
3. *Cl. Beijerinckii*, 9C, 4D
4. *Cl. pectinovorum*, 10C
5. *Cl. saccharobutyricum*, 11C, 3D

The names employed in this publication are those by which the cultures were received and their use does not imply acceptance by the authors either from the standpoint of nomenclature or classification. A later publication in this series, will deal with the physiology and classification of the butyl-acetone bacteria.

The strains used in the present work are well known in bacteriological literature and include cultures employed in the production of butyl alcohol and acetone. Strains isolated in this laboratory have not been included for this reason.

So far as the method of determining gelatinolysis is concerned, there is agreement among the three procedures employed in this work. The modified mercury-protein precipitation method has given very satisfactory results in our hands and is simple and convenient. The use of the culture-tube viscometer is likewise to be recommended. This method gives excellent results and provides for the determination of the progress of gelatinolysis with time. For cultures growing at 37° C. over a period of 48 hours, no rejuvenation is necessary. Before using the culture-tube viscometer at incubation temperatures below 37° C., it would be necessary to determine whether rejuvenation is required. Should rejuvenation be necessary at 50° C. for ten to twenty minutes, it would of course, prevent taking readings at intervals.

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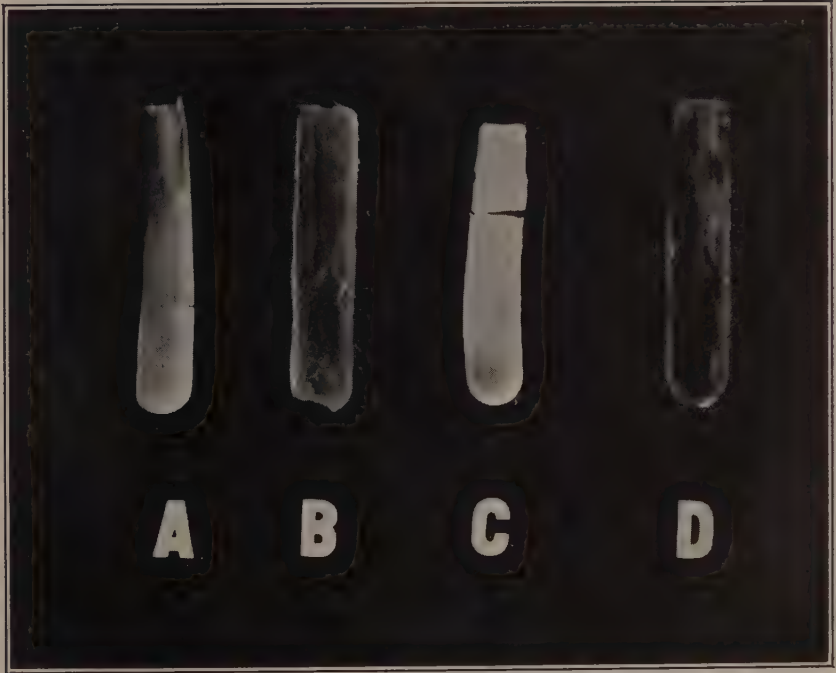
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Plate I

Gelatinolysis. Mercury-protein Precipitation Method.

- A. Control
- B. 13C,—*Cl. acetoniogenum*
- C. 3D,—*Cl. saccharobutyricum*
- D. 5D,—*Cl. felsineum*

Plate I



A SIMPLE, INEXPENSIVE ELECTRON TUBE POTENTIOMETER FOR USE WITH THE GLASS ELECTRODE

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The use of the hydrogen or quinhydrone electrodes with biological fluids such as blood-serum possesses serious limitations. The hydrogen electrode is poisoned easily by the protein materials and the quinhydrone measurements are seriously disturbed by oxidizing and reducing substances leading to drifting potentials and therefore to misleading results. The quinhydrone electrode also is limited in its use to values of pH less than 8. In studies on the properties of the hog cholera virus being made by one of the authors it was highly desirable to develop a method for pH determinations which would eliminate the difficulties noted above.

The glass electrode seemed to be the solution of the problem. In fact several investigators have demonstrated the utility of the glass electrode in the field of biology, but due to the very high resistance of the glass membrane, measurements cannot be made with the galvanometers and potentiometers commonly found in the laboratory. Until recently, electrometers of one type or another have been employed as null instruments. These are accurate and satisfactory in operation where the operator has considerable technical skill. These apparatuses have been elaborate, expensive, and have required much insulation and shielding for their proper operation. Many difficulties have been experienced even under most favorable conditions, to say nothing about those experienced under the average laboratory conditions.

With the advent of the radio and the development of electron tubes new tools have been placed in the hands of the scientific workers in the development of potentiometric methods. Recently several types of electron tube potentiometers have been developed which have been a decided improvement on the electrometer types and which are much less expensive. These investigators have contributed much toward the simplification and perfection of the glass electrode apparatus.

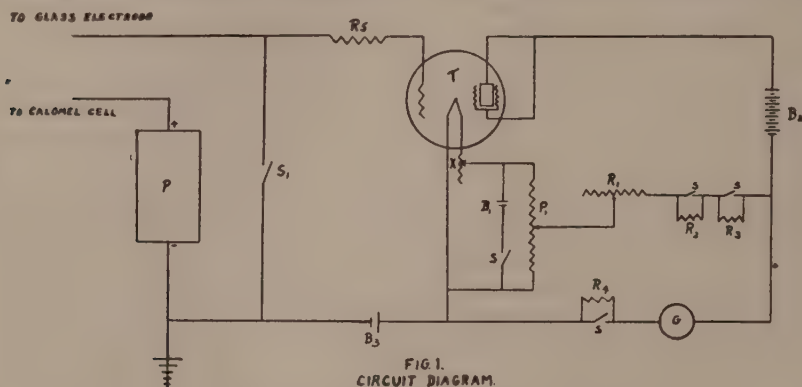
However these electron tube apparatuses have employed the more expensive tubes rather than the inexpensive electron tubes available at any radio shop. In this paper we shall describe a simplified instrument which employs the common inexpensive type of electron tube and which can be used with almost any type of potentiometer and fairly sensitive galvanometer. The apparatus is composed of standard units which can be readily obtained at relatively small cost. This makes it possible to convert a quinhydrone or hydrogen electrode apparatus to use with a glass electrode at a cost less than that of one of the electron tubes used in the earlier apparatus. The structure and operation are simple, being suitable for routine laboratory use. The apparatus can be made even more compact to form a portable unit. The wiring diagram is that of Harrison (1930) modified to employ an inexpensive electron tube which can be purchased at any radio supply shop.

A complete review of the development and use of the glass electrode will not be made at this time. However, the appended bibliography lists references not only concerning the glass electrode itself, but also dealing with the various units employed in such an equipment.

DESCRIPTION OF APPARATUS

Harrison (1930) points out two difficulties that arise when the common electron tube is used. First, the characteristic of the tube changes with a change of resistance in the grid circuit; and second, the common tube requires more current in the grid circuit to keep it in equilibrium than is furnished by the glass electrode. In this paper we wish to point out how these difficulties can be overcome and what common electron tube is most easily adapted.

The characteristic of a vacuum tube is the curve obtained when grid voltages as abscissae are plotted against plate current as ordinates. Resistance in the grid circuit shifts this curve. This change of characteristic necessitates a change in potential on the grid to keep the plate current



- B₁ — 2 VOLT STORAGE CELL
- B₂ — 22 1/2 VOLT "B" BATTERY
- B₃ — 1 1/2 VOLT DRY CELL
- G — GALVANOMETER
- P — POTENTIOMETER
- R — 400 Ω POTENTIOMETER
- R₁ — 10,000 Ω VARIABLE RESISTANCE
- R₂+R₃ — 24,000 Ω FIXED RESISTANCE
- R₄ — 1/4 MEGOHM FIXED RESISTANCE
- R₅ — 10 MEGOHM " "
- S — SWITCHES
- S₁ — GRID SWITCH
- T — UX232 ELECTRON TUBE
- X — 30 Ω FILAMENT CONTROL

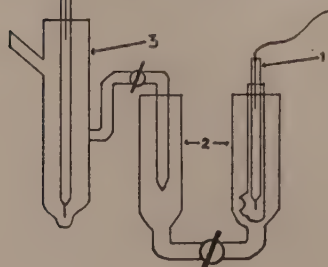


FIG. 2

- 1 — Ag-AgCl ELECTRODE
- 2 — KCl BRIDGE
- 3 — CALOMEL HALF CELL.

constant. We have overcome this difficulty by putting a large resistance in the grid circuit thereby changing the characteristic to the point where the addition of more resistance causes very little change in the characteristic. With this modification it is possible to measure cells with very high resistance with an accuracy less than one millivolt. Greater accuracy than this is not necessary with the glass electrode since each electrode is standardized separately against known buffers. The resistance of the electrode does not vary appreciably.

To overcome the second difficulty we have only to find the proper negative grid bias for a given tube. If the grid is positive with respect to the negative leg of the filament, a current will flow from the grid to the filament exactly as in the case of the plate. If the potential on the grid is zero, no current should flow, but usually there is a small leakage. To reduce this current, it is necessary to impose a negative charge on the grid. In the case of the UX 232 tube a "C" battery of one and one-half volts is entirely satisfactory. At this point the current from the grid to the filament is exceedingly small, about 1×10^{-10} amperes, if $22\frac{1}{2}$ volts are used on the plate. If more than 1.5 volts are used a current will flow in the opposite direction from that in the case just mentioned. One dry cell gives good results here. A flash light cell should not be used.

The switch S_1 of the diagram is kept closed unless a reading is being made. It will be noted that the potentiometer and cell are short circuited, but at balance no current will be drawn from the cell since the negative e.m.f. of the potentiometer just balances the positive e.m.f. of the cell to be measured. Also when the switch is kept closed the grid of the tube is connected through R_3 and through the "C" battery to the negative leg of the filament. This circuit is always constant so that as long as the switch is kept closed the grid potential is the same and the resistance from the filament to the plate in the tube does not vary; for example, suppose we have a cell we wish to read whose voltage is 0.20 volt and we tap the key S_1 when the potentiometer is set at zero. Instead of the grid being connected through the switch S' to the filament, it is connected through the cell and potentiometer. With the potentiometer at zero the potential on the grid is now different by 0.20 volt and the resistance from the filament to the plate inside the tube is varied proportionally. By adjusting the potentiometer to 0.20 volts the potential due to the cell in this circuit is neutralized and the potential on the grid is the same whether S_1 is closed or open. In practice the switch S_1 is opened and closed until no change is detected in the circuit containing the galvanometer.

In this hook-up using the UX 232 vacuum tube the screen grid and the plate are both connected to the positive pole of a $22\frac{1}{2}$ volt "B" battery. The filament current (0.06 amperes) is supplied by a 2 volt lead storage cell of large capacity. As soon as the filament is heated a current flows from the plate to the filament inside the tube. Suppose the circuit with the resistance R_1 , R_2 , and R_3 is left out for a moment. The current flowing in the plate circuit must go through the galvanometer G. The current is too strong for a sensitive instrument so that it is necessary to tap off an amount equal to this from the "A" battery and run it through the galvanometer in the opposite direction. In order to control this current, a potentiometer P_1 is connected across the "A" battery. This is not sufficient for fine adjustment so R_1 , R_2 , and R_3 are placed in the circuit. R_1 is variable and serves as a fine adjustment. R_2 and R_3 are provided with switches to short them out if necessary. It has been found that R_2 plus R_3

should equal 100,000 ohms and that the switches to short them out are not needed. The resistance R_4 is used to protect the galvanometer when the set is turned off and on. This is necessary because the filament takes some time to heat up while the compensating current starts immediately. As soon as the filament is heated the switch S may be closed and the compensating current adjusted so that the galvanometer stands at zero. This resistance is convenient when measuring an unknown e.m.f. where the galvanometer is likely to be damaged.

OPERATION

The filament of the vacuum tube is turned on and the rheostat turned up just to the point where the filament will be hot enough to function. Nearly all of the 30 ohm rheostat should be used. When this is once adjusted it seldom needs to be changed. Next adjust the galvanometer to zero with the switch S_1 closed. A standard cell is placed in series with a ten megohm grid leak in place of the glass cell and the potentiometer adjusted to the value of the standard cell. The apparatus is now ready for use. Measurements are made in the usual way as with any potentiometer. The vacuum tube merely acts as an amplifier for the galvanometer.

THE GLASS ELECTRODE

Electrodes were made according to the diagram from Corning glass No. .015. This is a very soft glass and great care must be exercised when the electrodes are blown. The end of the tubing is sealed off and a small bulb blown on the end. This bulb is heated very carefully in one spot on the side and drawn in. This membrane should be thick enough to withstand blowing with the mouth. These that can be blown out are too fragile to use.

The electrodes are filled with approximately 0.1N HCl and a silver chloride electrode is sealed in the top with paraffin. The silver-silver chloride electrodes are most conveniently prepared by the method of MacInnes and Beatty (1920). Electrodes prepared and sealed in this way have given good satisfaction for several months. They are kept in approximately 0.1N HCl when not in use.

METHOD OF CALIBRATION AND USE OF THE STANDARD EQUATION FOR THE GLASS ELECTRODE

As early as 1909 Haber and Klemenzievich showed that if two solutions at different values of pH each with a calomel electrode, are separated by a thin glass membrane, an e.m.f. is developed which may be related as,

$$\text{e.m.f.} = C(\text{pH}_2 - \text{pH}_1) \quad (1)$$

in which the constant C is practically equal to the expression RT/F found in the classic equations for chemical electromotive force. This means that the e.m.f. is a linear function of the difference in pH of two solutions.

In calculating the pH of a solution, using the glass electrode, the formula given by Stadie (1929) is efficient through ranges most commonly met in biological studies. For pH values higher than about 9.5 it is advisable to plot a curve using buffer solutions of known values, since the linear relation between pH and e.m.f. does not hold accurately for higher values of pH.

The equation is,

$$\text{pH} = \frac{NF}{kRT} (E - e_o) \quad (2)$$

in which,

E = the e.m.f. developed

e_o = a constant characteristic for a given electrode

The following data illustrate the determination of the values of the constants. Four buffered solutions were prepared and the values of pH carefully measured with the hydrogen electrode at 25°C. The values of the e.m.f. produced were determined under same conditions except that the glass electrode was substituted for the hydrogen electrode. The values were:

pH	e.m.f.
3.97	0.1528
5.97	0.2698
7.90	0.3839
9.90	0.4936

A graph of the values of e.m.f. against the pH values shows that the e.m.f. changes 0.0588 volts per unit change in pH, hence $NF/kRT = 0.0588$ and,

$$\text{pH} = \frac{E - e_o}{0.0588} \quad (3)$$

Substituting in equation (3) the values of E obtained at the various pH values and solving for e_o we obtain -0.0806 , hence, for the glass electrode used,

$$\text{pH} = \frac{E + 0.0806}{0.0588}$$

The value of the constant NF/kRT does not vary with the electrode but e_o is subject to some variation, hence it is advisable to check this constant each day against 0.05N potassium acid phthalate

SUMMARY

A simplified circuit has been described for an electron tube potentiometer for use with the glass electrode. The apparatus is composed of standard units which can readily be obtained at small cost. The equipment makes use of the common vacuum tube UX 232, which is inexpensive and can be obtained at any radio supply shop. Circuits previously described in the literature generally involve the use of the more expensive types of vacuum tubes, complex circuits, and elaborate shielding. The equipment described can be used with almost any type of potentiometer and fairly sensitive galvanometer and can be made compact to form a portable unit. The structure and operation are so simple that the apparatus is suitable for routine laboratory use

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THE LITERATURE OF ALKYLATED CARBOHYDRATES

VII. ALKYL DERIVATIVES OF DISACCHARIDES, POLYSACCHARIDES, GLYCOSIDES, AND MISCELLANEOUS SUBSTANCES

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INTRODUCTION

The previous papers in this series have described the derivatives of monosaccharides, namely, pentoses, mannose, galactose, fructose and glucose. In this paper the alkylated products of compound sugars, both natural and synthetic, the complex carbohydrates, the glucosides, and miscellaneous substances are described.

The notations employed in the earlier papers are continued. Names given in italics indicate the principal description of that compound. The figures appearing in parentheses, other than those possessing degree signs, refer to the numbered bibliography. For the lack of space, the arguments as to configuration and proper classification have not been included, and in most cases the compound has been described exactly as found in the literature. The final paper of the series will include all the publications which have appeared since the earlier papers were written, up to the time of publication of the final paper.

DISACCHARIDES

Lactose

Mention only of the important methylated derivative is made in 6, 7, 8, 11, 58, 61, 134 and 246. *Heptamethyl methyl lactoside* has a melting point of 77°-82° (10); 80° (69); 81°-82° (138) and 81.5°-82° (56). The minute colorless crystals, probably the beta-modification (10), are inactive toward buffered potassium permanganate (236), and is much faster acted upon by emulsin than tetra-methyl beta-methyl glucoside (69). For refractive indices and rotations, see 10, 56 and 138. The alpha-form is a colorless oil, of boiling point 210° at 1.1 mm. (10). From the lactoside may be prepared (102) an *octamethyl methyl lactobionate*, $[\alpha]_D = +34^\circ$ (101), which is characterized by a barium salt (102). This lactobionic acid may be broken down to 2, 3, 5, 6-tetramethyl gamma-glucenolactone and 2, 3, 4, 6-tetramethyl galactose (102, 103, 146). The 2, 3, 6-trimethyl glucose and 2, 3, 5, 6-tetramethyl galactose are the scission products of heptamethyl methyl lactoside (10, 22, 35, 50, 88, 91, 98, 118, 138, 156). See also 206 and 210. A *hexamethyl methyl lactoside* has been mentioned (10).

Sucrose

Octamethyl sucrose (1, 2, 17, 19, 39, 45, 49, 55, 64, 87, 131, 160, 221, 226) does not exhibit inversion on hydrolysis with dilute acids as it should (13). This rate of hydrolysis has been studied (179). The octamethyl sucrose forms tetramethyl glucose and tetramethyl fructose on hydrolysis (8, 40, 50, 131, 160, 208, 210, 221, 229, 247), while the *heptamethyl sucrose*

gives a 2, 3, 6-trimethyl glucose and a tetramethyl gamma-fructose (13, 41, 42, 43, 44, 46, 78, 99, 100, 208, 210, 247). The care with which the scission products of sucrose should be examined has been emphasized in the introduction to a previous paper (208).

The octamethyl derivative is a viscid syrup, b.p. 176° at 0.05 mm. (6, 7); $183^{\circ} - 6^{\circ}$ at 0.05 mm. (1, 2, 64, 87) and has a density at 20° of 1.1406 (6, 7). $n_D = 1.4588$ (6, 7) and $[\alpha]_D = +64.2^{\circ} \rightarrow +52^{\circ}$ (water) (1, 2, 64, 87). The heptamethyl derivative is a viscid syrup (6, 7), of boiling point 191.5° at 0.18 mm., and a density of 1.1654 (25°). The rotation is $+68.5^{\circ}$ (methyl alcohol).

It has been recently (247) claimed, however, that methylated sucrose (mixture of heptamethyl and octamethyl sucrose) gives a large number of hydrolysis products.

Gentiobiose

Methylated gentiobiose is mentioned in 95, and this compound served as a test substance for a new methoxyl micro-method (93). It is more accurately described as a *beta-methyl heptamethyl gentiobioside* (210). It has a melting point of 106° (64), does not reduce Fehling's (64), and has a reducing value of 12.1 if glucose is arbitrarily given (74) a value of 100. The rotation is stated (64, 238) to be $[\alpha]_D = -33.9^{\circ}$ (water). Tetramethyl glucose and 2, 3, 5-trimethyl glucose are the products of hydrolysis by dilute acids (57, 62, 111, 210). These are the same products as result from the hydrolysis of methylated maltose (92).

Melezitose

Methylated melezitose hydrolyzes to give 2, 3, 4, 6-tetramethyl glucopyranose and trimethyl gamma-fructose (94, 109, 208, 210, 229).

Maltose

When maltose is methylated, *octamethyl maltose* (heptomethyl β -methyl maltoside) is obtained (8, 11, 58, 97, 185). If acetoiodomaltose is methylated, the desired heptamethyl methyl maltoside is not obtained (12, 112), but a hexamethyl methyl gluco-arabinoside. A trimethyl glucose and a tetramethyl glucose result as the hydrolytic products (11, 12, 50, 92, 118, 156, 205, 210), which are the same as result from methylated gentiobiose.

The oxidation of maltose leads to an *octamethyl maltobionic acid* characterized by its *methyl ester* (75, 101, 103, 219). This ester may be prepared, furthermore, from maltobionic acid (75), trimethyl amylose, trimethyl glycogen and starch (219). Splitting of this methyl ester results in 2, 3, 4, 6-tetramethyl gluconic acid (75, 101, 103, 219).

Octamethyl maltose is a colorless syrup, distilling at $189-191^{\circ}/0.2$ mm., (1, 2, 75, 82, 111); $189-190^{\circ}/0.09$ mm., (64), soluble in light petroleum and water, and with a rotation variously stated between $+81.4^{\circ}$ in water to $+89.5^{\circ}$ in alcohol and acetone (64, 241).

Melibiose

Heptamethyl α -methyl melibioside are crystals, melting at $122-123^{\circ}$ (95). *Heptamethyl β -methyl melibioside* melts at $106-7^{\circ}$, and rotates $+97.8^{\circ}$ (water) (95). From this, may be prepared 2, 3, 4, 6-tetramethyl galactose and 2, 3, 4-trimethyl beta-methyl glucoside (95, 101, 204, 206, 210, 244). *Methyl octamethyl melibionate* (101, 244) forms 2, 3, 4, 6-tetramethyl galactose and 2, 3, 4, 5-tetramethyl gluconic acid on hydrolysis.

Cellobiose

Methylated cellobiose is discussed without mention of any physical properties in 22, 58, 61, 73, 105, 149, 177, 193, 211, 244 and 246. *Dimethyl cellobiose* and *dimethyl cellobiose octaacetate* have been described (187). A *hepta-methyl benzyl cellobioside* has been reported (174, 242) and also an *octaethyl cellobiose* (65, 105, 128 and 138). *Heptamethyl cellobiose-1-chlorohydrin* is a colorless syrup. The principal alkylated derivatives, however, are the octamethyl and the heptamethyl cellobiose.

Octamethyl cellobiose (244) may be prepared in the following ways:

- (1) From heptacetyl methyl cellobioside (138).
- (2) From diacetyl hexamethyl cellobiose (180).
- (3) Synthetically, by the combination of 1, 2, 3, 6-tetramethyl glucose and 1-chloro-2, 3, 4, 6-tetramethyl glucose (173, 242). The 2, 3, 6-trimethyl glucose and 2, 3, 4, 6-tetramethyl glucose are formed on hydrolysis (22, 25, 35, 50, 51, 118, 156, 202, 210).

Physical properties of the octamethyl cellobiose are:

B.P.	MM.	M.P.	Rotation	References
190-200°	.02	76-78°	$[\alpha]_D +9.0^\circ$ (water)	64
160-165°	.08	86°	$[\alpha]_{578}^{18} -15.5^\circ$ (water)	174
156-160°	.08			175
		86°	$[\alpha]_D^{19.5} -15.7^\circ$ (water)	138
			$[\alpha]_{578}^{18} -16.2^\circ$ (meth. alc.)	241
165°	.01	89-90°	$[\alpha]_D^{22} -17.0^\circ$ (water)	218
		87-88°		243

The x-ray diagram of this compound has been studied (128). The reducing value has been determined (74, 111) as 20.2 if glucose is 100. The methoxy groups may be conveniently determined by a simple micro-method (93). The octamethyl cellobiose which is the beta-form, may be oxidized (101, 244) to *methyl octamethyl cellobionate*. See reference (103) for a description of this compound.

The *heptamethyl cellobiose* may be prepared (174, 242) from the octamethyl cellobiose. It consists of needles, m.p. 105-110°, of rotation $+40^\circ$ in water (174). The boiling point is reported to be 180-185° at 0.1 mm. (242).

Methylated celloside (cellobioside) is said (24, 39, 225, 237) to give 2, 3, 5, 6-tetramethyl glucose and 2, 3, 6-trimethyl glucose.

Trehalose

The rotation of the *octamethyl* derivative is given as $[\alpha]_{578}^{18} = +52^\circ$ (3% methyl alc.) (174, 176). 2, 3, 4, 6-Tetramethyl glucose is the hydrolytic product (170).

Turanose

This disaccharide forms an *octamethyl* derivative (94, 208). Trimethyl fructose results from this on hydrolysis and this, in turn, forms dimethoxyhydroxy glutaric acid (94).

Synthetic Disaccharides

The preparation of 1- β -methyl heptamethyl-6- α -glucosido-glucose, $[\alpha]_D +93.1^\circ$ in water, has been described (238). 4-Glucosido-mannose (210,

216) forms *heptamethyl-4-β-glucosido-α-methyl mannoside* (209, 210, 214) of boiling point 177-180° (216) and rotation +28° in water. This compound may be broken up to tetramethyl glucose and trimethyl mannose. The 4-glucosido-mannose may be oxidized to 4-glucosido-mannonic acid, and when this is methylated, *methyl octamethyl-4-glucosidomannonate* is produced. This consists of square plates, melting at 118°.

The analogous galactose compound, 4-galactosido-mannose will give 4-galactosido-mannonic acid and this, in turn, will methylate to *methyl octamethyl galactosidomannonate* (a bionic ester). This has been broken down to the hydrolytic products. *Heptamethyl-4-β-galactosido-α-methyl mannoside* is a colorless viscid syrup, boiling at 170°/0.02 mm. (209, 210, 214).

Miscellaneous Disaccharides

Derivative	Remarks	References
Difructose anhydride	Hexamethyl ester	244
Dimethyl diketose		250
Hexamethyl biosan	Gives 2, 3, 6-trimethyl glucose	79, 128, 137 210
Tetramethyl biosan		163
Hexamethyl amylobiose	Scission products	89, 209, 210
Hexamethyl methyl glucoarabinose		205, 210
Disaccharide from 2, 3, 5, 6-tetra- methyl galactoside	Non-reducing	206

TRISACCHARIDES

Cellotriose

Decamethyl beta-methyl cellotrioside (210, 212, 213, 237, 241, 244) may be prepared by the acetolysis and methylation of cellulose (175). On hydrolysis, it breaks up to give tetramethyl glucopyranose and 2, 3, 6-trimethyl glucose (218). The melting point is given as 115° (174, 176); 117° (242); 117-8° (218); 118° (175). It distills at 216-220°/0.08 mm. (174, 175, 176). Its rotation in sodium light in methyl alcohol at 18° is -14.5° (218). See 174, 175 and 176 for rotations in other solvents.

Maltotriose

The *hendecamethyl* derivative has been mentioned (213, 241).

Raffinose

Hendecamethyl raffinose is a viscid syrup (43, 44), distilling at 238-240° at 0.02 mm., with $N_D = 1.4680$ and $[\alpha]_D = +128.4^\circ$ (water). Normal tetramethyl galactose (2, 3, 4, 6), 2, 3, 5-trimethyl glucose and 1, 3, 4, 5-tetramethyl γ-fructose are scission products, (43, 44, 95, 111, 206, 208, 210).

TETRASACCHARIDES

Cellotetrose

Along with the triose compound, *tridecamethyl β-methyl cellotetroside* (210, 212, 213, 237, 241, 244) is said to be formed during the simultaneous acetolysis and methylation of trimethyl cellulose (218). It is also possible that it might be a methylated cellodextrin. In physical properties it is said to consist of irregular prisms, melting point 139° (175); 151-2° (218), with a boiling point of 265-275° at 0.1 mm. The rotation is $[\alpha]_D^{18} = -10^\circ$ (water) (218).

Maltotetrose

A *tetradecamethyl* compound has been described (213, 241).

POLYSACCHARIDES

Starch and Glycogen

Space does not permit the recording of the many patents covering the alkyl and aralkyl ethers of starch and glycogen. Methods of alkylation will be found detailed in 120, 130 and 143. Constitutional studies will be found in the references 155, 189, 191 and 192. Methylated starch or glycogen are discussed in 21, 24, 72, 83, 96, 107, 113, 143, 172, 213, 241 and 246.

Dimethyl starch is stated (120) to have a rotation of $+143^\circ$ in chloroform. *Tetramethyl starch* is said (38, 39) to be easily soluble in cold water, alcohol and many other organic solvents. Its molecular weight in water and phenol appears to be between 900-1200 (38).

Trimethyl starch is the best known compound. It is a white powder, does not reduce Fehling's solution, and is soluble in the usual organic solvents (157). It has a melting point of 145° (120); 147° (157) and rotates $+208^\circ$ in chloroform at 20° (120, 157). On oxidation, methyl octamethyl maltobionate is formed (219).

On hydrolysis, trimethyl starch or glycogen produces a 2, 3, 6-trimethyl glucose (50, 56, 58, 61, 107, 155, 157, 209, 210 and 219) and a dimethyl glucose (28, 32, 33, 120, 143, 159). It is claimed (256) that glycogen acts in a different manner toward methylating and acetylating agents than does starch.

Xylan

Xylan, treated with dimethyl sulfate and sodium hydroxide, forms a mixture of *monomethyl xylan*, *dimethyl xylan*, and *unmethylated xylan* (154). *Acetyl methyl xylan* has been described (198). *Dimethyl xylan* (30, 52, 205) is a white solid, $[\alpha]_D = -92^\circ$ (chloroform), insoluble in water, stable to alkali and non-reducing (154). Haworth and Percival (220) give its preparation. Tasuku (198) finds its rotation to be -94.1° . A *methylated bionic acid* derivative may be obtained (220), which further leads to a *methyl ester of hexamethyl dixylo-bionic acid* (220).

Levan

Levan, considered as a 2, 6-anhydrofructofuranose (225), is the result of the metabolism of *Bacillus mesentericus* (226). From it may be prepared a *trimethyl levan*, which results in 1, 3, 4-trimethyl fructose (208) on hydrolysis. *Yucca dilevan* is said to form 3, 4, 6-trimethyl h-fructose (232).

Inulin

Methylated inulin is mentioned in 36, 72, 114, 140, 155, 167 and 253.

The partial methylation of inulin results in a *dimethyl inulin* (17, 140), of a levo rotation, and is described as a colorless amber syrup, becoming brittle on drying at 100°C . (17). It gives an opalescent solution in hot water, and, on hydrolysis, leads to the formation of a dimethyl gamma-fructose (32, 33). The dimethyl inulin may be further treated with methylating agents to give a levo or dextro *trimethyl inulin* (17, 37, 40, 42, 121). Trimethyl gamma-fructose is the hydrolytic product resulting from the scission of this trimethyl inulin (17, 32, 33, 37, 40, 42, 50, 87, 107, 119, 121, 155, 162, 169, 208). The properties of the trimethyl

inulin are described by Karrer and Lang (23), and by Irvine and Steele (17). The latter authors describe it as a colorless syrup, soluble in alcohol, chloroform, and sparingly soluble in ether. These solubilities are supported by (38), who further add that the product is more soluble in cold water than in hot. The melting point is recorded as 138° - 140° (121, 258). The rotation is stated to be $[\alpha]_D^{15} = +50.34^{\circ}$ (alcohol.) (17) and $[\alpha]_D^{20} = -50.2^{\circ}$ (Bz) (121). See (114) for mention of *triethyl inulin*.

Amylose

Methylated amylose (209, 210) is said to give a tetramethyl glucose (245) and 2, 3, 6-trimethyl glucose (161). *Trimethyl amylose* may be converted to methyl octamethyl maltobionate (219). The action of thionyl chloride on *methylated alpha-tetra-amylose* ($[\alpha]_D = +152^{\circ}$) is discussed (161). *Hexa-[trimethyl amylose]* (161) and *tetra-[dimethyl amylose]* (26, 161) are mentioned.

Cellulose

Trimethyl cellulose is mentioned, but not described, in references 4, 5, 9, 10, 14, 16, 17, 22, 29, 31, 38, 42, 47, 48, 51, 59, 60, 63, 66, 67, 72, 77, 79, 80, 83, 84, 88, 96, 104, 107, 110, 113, 115, 116, 123, 125-7, 132, 134, 137, 144, 145, 148, 150, 152, 158, 161, 164, 166, 171, 172, 182, 186, 194, 196, 197, 200, 213, 224, 241, 244, 246, 251, 252, and triethyl cellulose in 105, 106, 128, 132, 164, 171, 186, 197 and 251. The references given do not include the many patents on alkylated and aralkylated celluloses which may be found in the recent patent literature. A review of some recent patents and literature on new derivatives of cellulose may be found in the series of articles by Trotman (199), Mullin (251, 252), Montonna (Paper Trade J., 86: 61-70 (1928), and by Stark (259).

For the methylation and ethylation methods, see the following:

- (1) General—4, 130, 139, 141, 147, 187, 217, 223, 251, 252.
- (2) Copper-ammonium cellulose—68, 251, 252.
- (3) Viscous cellulose—68, 251, 252.
- (4) Cellulose dextrin—68.

Complete information on the x-ray studies of alkylated cellulose may be found in 127, 128, 168, 183, 188, 222 and 235. The chemistry of the important methyl derivatives is discussed in 124, 155, 192, 201 and 235. The action of ultra-violet light on cellulose derivatives is given in 254.

Some of the less common alkylated derivatives of cellulose are:

- (1) *Dimethyl cellulose* (68, 145, 200).
Color reactions with zinc chloride and iodide are discussed (135).
- (2) *Diethyl cellulose* (135).
- (3) *Monomethyl dicellulose* (133, 187, 209).

This compound, prepared from sodium cellulose diethiocarbonate (165), exhibits no viscous reactions.

- (4) *Monomethyl cellulose* (68).
- (5) *Dimethyl hydrocellulose* (68).
- (6) *Hydroxymethyl cellulose ether* (231).
- (7) *Benzyl cellulose* (228).

This compound is now on the market.

Numerous derivatives are described in patents. Cellulose was the first

carbohydrate methylated by the dimethyl sulfate method (51). Simultaneous acetolysis and methylation of trimethyl cellulose (218) gave a mixture: (a) Tetramethyl methyl glucopyranoside, (b) Heptamethyl β -methyl cellobioside, (c) methylated cellodextrin and (d) Hendecamethyl cellotriose. Some tetramethyl methyl glucopyranosides are probably secured in addition to trimethyl methyl glucosides when trimethyl cellulose is hydrolyzed (245), but there is no doubt but that 2, 3, 6-trimethyl glucose is the only trimethyl glucose secured from completely methylated cellulose (9, 18, 20, 21, 22, 28, 32-35, 51, 56, 58, 61, 71, 108, 110, 117, 119, 136, 142, 151, 153, 155, 159, 209, 210, 217, 219, 224, 245).

Trimethyl cellulose

Methylated cotton is a white, friable powder which still preserves the fibrous nature of the original material (90, 108). It forms with cold chloroform and acetylene tetrachloride, a highly viscous clear solution, insoluble in water, alcohol, ether, acetone, and other solvents. It exhibits a change of dispersion in chloroform and tetrachlorethane (90, 117, 195, 222). The theoretical methoxyl content is 45.5% (14), but the highest obtained is that of 44.7% after 26 methylations (117, 141). Trimethyl cellulose is not identical with 2, 3, 6-trimethyl glucose anhydride in any respect (212). The products of hydrolysis of the repeatedly methylated cellulose included mono-, di-, and trimethyl glucoses (27). A xanthate with sodium hydroxide and carbon disulfide is formed by the product of the second methylation (4). Representative rotations (benzene) are: -18.5° (20°) (105); -18.37° (20°) (129); -18° (120°); -18.58° (21°) (176). The methylated cellulose is said (135) to give distinctive color reactions. In an ether solution of hydrochloric acid, a 2, 3, 6-trimethyl-1-chloroglucose is formed which is recognized by a readily crystallized pyridium salt (117).

Triethyl cellulose

This alkylated derivative of cellulose has the same solubilities as the methyl compound (147), and exhibits the same change of state of dispersion in certain solvents (222). It is stated (147) that cellulose cannot be ethylated (234) in the presence of pyridine or magnesium hydroxide by the ethyl chloride method. The ethyl cellulose is stable in hot alkali solution (147). The viscosity has been measured (233). It is possible to prepare the acetylated derivative from the ethylated cellulose (15). Triethyl cellulose exhibits a rotation of $+49.1^\circ$ (105) and $+49.4^\circ$ (127) in pyridine, and $+26.7^\circ$ in benzene (127). The substance practically melts at 260° , and then decomposes slowly.

Cellan

Methylated cellan is claimed to give 2, 3, 4, 6-tetramethyl glucose.

Cellodextrin

The composition of this substance is in doubt. The physical properties indicate the possibility of it being a tridecamethyl cellotetrose. It consists of irregular prisms, melting at $151-2^\circ$ (218). $[\alpha]_D^{25} = -10^\circ$ (water).

Gluco-Heptose

A methylated gluco-mannan may be prepared by the action of alkylating agents on Konjak (190). The 2, 3, 4-trimethyl glucose and 2, 3, 6-trimethyl mannose are scission products (210).

ALKYLATED HEPTOSES

Sedosan

Sedosan, when methylated, forms a *tetramethyl sedosan* (184). When this is treated with nitric acid, optically inactive trimethoxyglutaric acid is formed.

Gluco-Heptose

From 3-methyl glucose, by the cyanohydrin synthesis, *4-methyl glucoheptose* may be prepared (248). The sugar has an indefinite melting point, and its rotation at equilibrium is -15° in water. It may be oxidized to *4-methyl glucoheptonic acid* (70) which, when allowed to stand in water forms only a 5-ring lactone (86). The *4-methyl glucoheptonic lactone* may be identified by its *osazone*, m.p. 160° (248).

A *beta-pentamethyl α -glucoheptose* has likewise been announced (215, 248). The transparent monoclinic prisms melt at 84° . As a syrup it boils at $157^\circ/0.18$ mm. The optical rotation at equilibrium is -42.5° (215); -44.5° (248). It reduces Fehling's solution.

A *pentamethyl beta-methyl alpha-glucoheptoside*, non-reducing, has been prepared as a colorless oil, levo-rotatory, and with a refractive index of $N_D^{17} = 1.4487$ (215). The corresponding lactone, 2, 3, 4, 6, 7-*pentamethyl α -glucoheptonic* lactone, is crystalline, melting at 83° , and with an equilibrium rotation of $+9.4^\circ$ (38 hours). This lactone may be broken down to methyl i-dimethoxysuccinate, m.p. 68° .

GLYCOSIDES

Mention of the following methylated derivatives may be found in the literature.

Derivative	Remarks	References
Tetramethyl indican		40, 210
Monomethyl lichenin	2, 3, 6-trimethyl glucose	59, 71, 72, 210
Dimethyl salicin	Prisms, m.p. 122°	6, 7
Pentamethyl salicin		3
Pentamethyl arbutin	Tetramethyl glucose	54, 210
Monomethyl chitosan		187
Methylated aesculin	Yellow glass, solubilities	210, 227
Methylated sinistrin		208
Paullinia tannin		210
Vegetable ivory		207

The literature of the heart specific glycosides, which contain naturally occurring alkylated carbohydrates of debatable structure, will be discussed in the final paper of the series.

Amygdalin

Amygdalin, after methylation (95, 210), may be broken down to a 2, 3, 4, 6-tetramethyl glucose and a 2, 3, 5-trimethyl glucose (28, 53). The *heptamethyl amygdalinic acid methyl ester* has a melting point of 91° (64, 81) and exhibits a rotation of -49.3° . It is almost insoluble in water.

MISCELLANEOUS

Name	Remarks	Reference
Methylated bornyl d-glucuronide	d-dimethoxysuccinic and i-xylotrimethoxyglutaric acid	230

Methylated theophyllin		
l-arabinoside	d-arabotrimethoxyglutaric acid	230
Trimethyl N-methyl		
adenosine HCl	Gives 2, 3, 5-trimethyl ribose	249
Methyl trihexosan	Doesn't reduce Fehling's	85, 96, 122, 120
Methylated dihydro-		
sphingosine		206

Methylated or ethylated mesquite gum results (239) in the formation of the partially alkylated lactone of methoxy d-glucuronic acid combined with three molecules of d-galactose.

The methylation of gum arabic (203) results in the preparation of methyl heptamethyl aldobionate (the methyl ester of hexamethyl glucuronosidomethyl galactoside). See the original paper for physical and chemical characteristics of both the alpha and beta forms of this substance. 2, 3, 4-Trimethyl glycuronic acid is the hydrolytic scission products.

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THE LITERATURE OF ALKYLATED CARBOHYDRATES

VIII. ACCUMULATED LITERATURE SINCE PUBLICATION OF EARLIER PAPERS

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INTRODUCTION

Many references dealing with alkylated carbohydrates have been collected since publication of the earlier papers of this series. In this, the last of the series, these later references are summarized and brought up to the date appearing under the title. Only the new compounds, which were not described previously, are italicized and, as before, signify the principle description of the compound. The author's nomenclature is retained unless otherwise stated, and whenever there is any doubt as to the structural position of the alkyl groups, the compound is described under the several headings in question.

TRIOSE, TETROSE AND PENTOSE DERIVATIVES¹

TRIOSES

Glycerol

Papers have been published within the last year on various monomethyl ethers (30, 54, 69, 79, 89, 100), dimethyl ethers (18, 30, 42, 54, 100, 101, 115, 134) and trimethyl ethers (19, 47, 54). The α , β -dimethyl glycerol was prepared pure for the first time (54) by the action of dimethyl sulfate on sodium glyceroxide. The α , γ -dimethyl glycerol was not fermented by a selected group of organisms (42). Diethyl glycerin (54, 100) and monomethyl glycerin (54) have been prepared. The 3-methyl ether of glycerin-aldehyde, prisms, melting point 120-1°, may be used (79, 89, 102) for the preparation of the 3-methyl ether of dihydroxyacetone. Previous literature in this field has been discussed (100).

Diphenyl Triose

A monomethyl ether of a diphenyl triose, which may be described as alpha-hydroxy-beta-methoxy-beta-phenylpropioiphenone, has been briefly described (80).

TETROSES

It is said (72) that probably a dimethyl tetrose is formed by the action of alkali on the trimethoxy-urethanes produced when the isomeric arabonamides are treated with alkaline hypochlorite.

Succinic Acid Derivatives

Dimethoxy succinic acid is the degradation product of 2, 3-dimethyl methyl glucoside (123), 2, 3, 4-trimethyl glucuronic acid (86), pentamethyl α -glucoheptono- δ -lactone (59) and methylated bornyl d-glucuronide (84). This dimethoxysuccinic acid may be identified as the diamide (84, 86, 123).

¹ Iowa State College J. of Science 5:61-69 (1931)

The methyl amide of inactive dimethoxysuccinic acid has a melting point of 208° (59).

PENTOSES

Rhamnose

Gamma-monoacetyl-3, 4-dimethyl methyl rhamnoside, which is unstable in acid solution, results in trimethyl methyl rhamnopyranoside, distilling at 79°/0.02 mm., and with $N_D^{20} = 1.4440$ (58). The 2, 3, 4-trimethyl rhamnonic acid has been mentioned (33), and the conductivity of trimethyl rhamnonolactone studied (32, 76). The physical characteristics of 1-2, 3, 4-trimethyl rhamnonolactone (m.p. 40-4°) and 1-2, 3, 5-trimethyl rhamnonolactone (m.p. 75-76°) have been determined (35, 36). The 5-methyl rhamnose phenylhydrazone (65) melts at 163-4°.

Ribose

The trimethyl ribose from adenosine differed from the trimethyl d-ribose derived from the normal methyl d-riboside and hence did not possess the pyranose structure (133). Ribose derivatives are:

(a) 2, 3, 4-Trimethyl methyl riboside (76).

A mobile liquid, boiling point 54°/0.05 mm., with $[\alpha]_D^{24} = -35^\circ$ (H₂O).

(b) 2, 3, 4-Trimethyl ribose (76, 158).

Melts at 85°; rotation at 27° is -40° (final). Prepared from (a) (76).

(c) 2, 3, 4-Trimethyl delta-ribonolactone (38, 76).

Prepared from trimethyl arabinolactone by the process of epimerization (38) or from (b) (76). Its rate of hydrolysis is similar to that of trimethyl delta-rhamnonolactone (76). The boiling point is 93-95°/0.05 mm., and rotation $+85.4^\circ$ (benzene). Oxidation results in i-ribotrimethoxyglutaric acid, identified as the dimethyl ester, b.p. 77-78°/0.02 mm., which is identical with the product secured from tetramethyl sedosan (38).

(d) 2, 3, 5-Trimethyl ribose (132, 153, 158).

The hydrolytic product of methylated guanosine (132) and adenosine (153, 158). The substance boils at 90-92° at .02 mm., and rotates $+51.6^\circ$ in water at 26° C., and $+41.4^\circ$ in absolute alcohol. From this ribose may be prepared dimethyl i-dimethoxysuccinate (m.p. 68°) (132, 153) which is the same as the preparation from mesotartaric acid.

(e) 2, 3, 5-Trimethyl gamma-ribonolactone (153).

Boiling point is 110-115° at 0.2 mm.; $N_D^{20.5} = 1.4501$.

Lyxose

Trimethyl lyxoses (33) are prepared (78) by the epimerization of the corresponding trimethyl xyloses. Dextro-2, 3, 4-trimethyl lyxonolactone (35) is a liquid, $+35.5^\circ$ (water), -87° (ether) (36), giving a d-trimethoxyglutaric acid dimethyl ester (56, 76) when treated with nitric acid. The corresponding dextro-2, 3, 5-trimethyl lyxonolactone (35) has a melting point of 44°, rotating $+82.5^\circ$ (water) (36), and is similar to the trimethyl gamma-ribonolactone in rate of hydrolysis (153). A trimethyl lyxonic acid brucine salt is reported (56).

Arabinose

Sodium 2, 5-dimethyl d-arabonate was found not to be fermented by certain organisms (42). Derivatives of 2, 3, 4-trimethyl l-arabinose <1, 5>, the normal form, which have been described recently, are:

(a) 2, 3, 4-Trimethyl delta-arabonolactone (37).

Needles, m.p. 45° (23, 36, 97), hygroscopic in the liquid form, with an optical rotation of $+180^{\circ}$ (65), $+181^{\circ}$ (H_2O) (36). Conductivity measurements have been reported (32, 35). The lactone is polymerized by hydrochloric acid (23, 97) to give the compound melting at $135\text{--}138^{\circ}$. As shown in the earlier paper, it results in trimethoxyglutaric acid (76).

(b) 2, 3, 4-Trimethyl arabonamide.

This substance melts at 96° . It exhibits a dextro-rotation of $+35.5^{\circ}$ (alcohol) and $+25.7^{\circ}$ (water). Alkaline hypochlorite produces trimethoxyurethanes.

(c) Trimethylarabonic acid brucine salt (78).

(d) Trimethyl beta-methyl l-arabinoside.

A melting point of $81\text{--}82^{\circ}$ and rotation of $+158^{\circ}$ at 25° is recorded.

(e) 2, 3, 4-Trimethyl alpha-methyl L-arabinoside.

This derivative melts at 44° (23), $46\text{--}46.5^{\circ}$ (78) with a rotation of $+25^{\circ}$ (65). It is not fermented by certain organisms (42).

The 2, 3, 4-trimethyl l-arabinose $<1, 5>$ is given a melting point of $81\text{--}82^{\circ}$ (78), with $[\alpha]_D^{25} = +158.0^{\circ}$ (78). Fermentation properties have been published (42). A detailed account of the preparation of this sugar is given by Neher and Lewis (78).

The following derivatives of 2, 3, 5-trimethyl arabinose have been reported lately:

(a) 2, 3, 5-Trimethyl arabonamide.

M.p. 132° ; $[\alpha]_D = +19.3^{\circ}$ (alcohol) (72). Results in trimethoxyurethanes when treated with alkaline hypochlorite.

(b) 2, 3, 5-d-Trimethyl gamma-arabonolactone $<1, 4>$ (107).

This compound $[\alpha]_D = +44^{\circ}$ (24) can be transformed to trimethyl delta-ribonolactone by the process of epimerization, but with difficulty (38). The arabonolactone may be prepared from trimethyl 2-keto gluconic acid (107), or from tetramethyl γ -fructose (24). It gives rise to l-dimethoxy tartaric acid (107).

(c) 2, 3, 5-Trimethyl l-arabonolactone (37, 107).

Melts at 33° ; $[\alpha]_D = -9^{\circ}$ (chloroform); -44° (water) (36). See (32, 35) for conductivity measurements. May be prepared from the parent sugar, 2, 3, 5-trimethyl arabinose (36).

Xylose

The epimerization of trimethyl xylose to the corresponding lyxose compound has been successful (78). The oxidation of normal derivatives of trimethyl xylose is said to give trimethoxyglutaric acid in every case (76).

Alkylated xylose compounds mentioned in recent literature are:

(a) 2, 3, 4-Trimethyl xylopyranose (normal) (107).

A very pure sample has a rotation of $+17.8^{\circ}$ in water (56) and $+21.3^{\circ}$ (methyl alcohol). It melts at $90\text{--}92^{\circ}$. The sugar can be prepared from the methyl ester of hexamethyl dixylobionic acid (62). On oxidation it forms

(b) 2, 3, 4-Trimethyl xylonic acid (56).

This acid may be separated from the corresponding lyxonic acid by fractional crystallization of their brucine salts (56).

(c) 2, 3, 4-Trimethyl β -methyl xyloside.

Certain organisms were not capable of fermenting this sugar (42).

(d) 2, 3, 4-d-Trimethyl δ -xylonolactone (32, 35). Melts at 56° ; $[\alpha]_D = \pm 9^\circ$ (chloroform) (36).

(e) 2, 3, 5-Trimethyl xylose (42).

(f) 2, 3, 5-Trimethyl gamma-methyl xyloside (42).

(g) 2, 3, 5-Trimethyl xylonic acid (62).

(h) 2, 3, 5-Trimethyl xylono phenylhydrazide. The melting point is given as 88° (62).

(i) d-2, 3, 5-Trimethyl γ -xylonolactone (35, 36, 107).

Obtained by hydrolysis of the methyl ester of hexamethyl dixylobionic acid (62). It is a pale yellow syrup, distilling at, $80-90^\circ/0.05$ mm. $N_D^{16} = 1.4450$, and $[\alpha]_D^{17} = +70^\circ$ (20 days-water) (62). See (32) for conductivity measurements made on this lactone.

Glutaric Acid Derivatives

Inactive-xylotrimethoxyglutaric acid is the result of the oxidation of methylated bornyl d-glucuronide (84), tetramethyl delta-gluconolactone, and 2, 3, 4-trimethyl glucose (51, 107). No xylotrimethoxyglutarate could be recognized in the oxidation products of 2, 3, 4-trimethyl glucuronic acid (86). The alkylated inactive glutaric acid may be identified by its diamide or by its methyl amide (m.p. $167-168^\circ$) (51).

The d-arabotrimethoxyglutaric acid can be isolated from the degradative products of methylated theophyllin l-arabinoside (84), and of trimethyl fructuronic acid (107). It is recognized from its crystalline diamide (84). The l-form is one of the products produced by the oxidation of pentamethyl α -glucoheptono- δ -lactone (59), and the crystalline methyl amide (m.p. 171° ; $[\alpha]_D^{19} = +60^\circ$) serves to identify the l-arabotrimethoxyglutaric acid (59). Octamethyl turanose (H. Urban-Cellulosechemie 7:73-81 (1926) is hydrolyzed to 1, 3, 4-trimethyl fructose which, in turn, leads to dimethoxyhydroxy glutaric acid, rotating at $+34.5^\circ$.

Inactive-ribotrimethoxyglutaric acid is recognized from the dimethyl ester (76) which distills at $77-78^\circ/0.02$ mm. It results, too, when tetramethyl sedosan is oxidized.

Further descriptions of the trimethoxyglutaric acids will be found in the text throughout this paper.

GALACTOSE DERIVATIVES

(Iowa State College J. of Science 5:71-75 (1931)

Monomethyl Galactoses

It is claimed (141) that the 4-methyl d-galactose of Pacsu and Lob (29) is probably a 2-methyl galactose. The 4-methyl d-galactose (33, 141) melts at 118° , and exhibits an optical rotation of $+67.8^\circ$ (3 hrs.-water) (29) at 18° C. It forms an osazone, m.p. $194-5^\circ$ (decomp.) (29). A 5-monomethyl galactosazone is mentioned (65). Monomethyl d-galactose benzyl mercaptal is reported to melt at $130-131^\circ$, and has a rotation of $[\alpha]_D^{13} -27.55^\circ$ (pyridine) (29). Diacetone-6-methyl galactose was not fermented by a selected group of organisms (42). The 6-methyl galactose is said to melt at 128° (29, 65). It forms a 6-methyl galactosazone, the melting point of which is variously reported as 177° (65) and 204.5° (29).

Trimethyl Galactoses

Those reported in the recent literature are the following:

(a) *2, 3, 4-Trimethyl galactose*.

A syrup which reduces Fehling's, $N_D^{18} = 1.4724$, and $[\alpha]_D^{20} = +83^\circ$ (water) (50). Forms 2, 3, 4, 6-tetramethyl galactose.

(b) *2, 3, 4-Trimethyl delta-galactonolactone*.

A viscoid syrup. Prepared from (a).

(c) *2, 3, 4-Trimethyl galactose phenylhydrazide*.

From (b). Needles melting at 165.7° (50).

(d) *2, 3, 4-Trimethyl mucic acid* (50).

Forms a *dimethyl ester*, plates, neutral to litmus of melting point 98° (50); 165.6° (13).

(e) *3, 4, 6-Trimethyl monoacetone galactose*.

Doesn't reduce Fehling's. $[\alpha]_D^{20} = -31.4^\circ$ (meth. alc.) (74).

(f) *3, 4, 6-Trimethyl galactose*.

A reducing liquid of $[\alpha]_D^{20} = -4.3^\circ$ (meth. alc.) (74).

(g) *3, 4, 6-Trimethyl delta-galactonolactone*.

Sodium salt — $[\alpha]_D^{25} = +29.4^\circ$ (water) (74).

Tetramethyl Galactoses

Derivatives containing four alkyl groups are described as follows:

(a) *2, 3, 4, 6-Tetramethyl delta-galactose*.

Preparation of this sugar has been successfully accomplished from methylated manninotronic acid (140), methyl octamethyl galactosidomannanate (57), and 2, 3, 4-trimethyl galactose (50). The melting point is $68-69^\circ$ (57).

(b) *2, 3, 4, 5-Tetramethyl mucic acid*.

Dimethyl ester — m.p. 103° ; *diamide* — m.p. 276° (13).

(c) *2, 3, 4, 6-Tetramethyl galactose anilide*.

The melting point is 193° (140), 195.6° (57).

(d) *2, 3, 4, 6-Tetramethyl α -methyl galactoside* (107).

Additional publications report the rotation to be $[\alpha]_D^{22} +96.5$ (72) and $+143.4^\circ$ (65).

(e) *2, 3, 4, 6-Tetramethyl β -methyl galactoside*.

Prepared from 2, 3, 4-trimethyl galactose (50). The product melts at 44.5° (50) and $[\alpha]_D = +20^\circ$ (water) (50) and $+30.7^\circ$ (65).

(f) *d-2, 3, 4, 6-Tetramethyl galactonolactone*.

A liquid, $+143^\circ$ (65); $+153^\circ$ (water) (35, 36); $+25^\circ$ (17 hrs.) (72).

(g) *d-2, 3, 4, 6-Tetramethyl galactonoamide*.

Results from (f). M.p. is 120° , $[\alpha]_D +37.9^\circ$ (72).

(h) *d-2, 3, 5, 6-Tetramethyl galactonolactone* (35, 36, 72).

(i) *2, 3, 5, 6-Tetramethyl methyl galactofuranoside* (72).

(j) *d-2, 3, 5, 6-Tetramethyl galactonoamide*.

Melts at 153° (decomp'n.); $[\alpha]_D +6.53^\circ$ (water) (72).

MANNOSE DERIVATIVES

(Iowa State College J. of Science 5:77-83 (1931))

Monomethyl Mannoses

3-Methyl glucose and 3-methyl fructose can form 3-methyl mannose through their common enol form (135). The 4-methyl mannose has been mentioned (29, 33). Paesu (141) discusses the question of the *4-monomethyl mannose* reported by him (28) and concludes that it must be a

2-methyl mannose. Assuming that Pacsu's (28) 4-methyl mannose compounds are, in reality, 2-methyl mannose compounds, he mentions in his paper:

(a) *2-Methyl mannose*.

A colorless thick syrup. $[\alpha]_D^{20} = +7.38^\circ$ (water) (28).

(b) *2-Methyl-d-mannose hydrazone*.

Colorless needles, m.p. 179° (28, 65).

(c) *2-Methyl d-mannose dibenzyl mercaptal*.

A thick syrup, recrystallized m.p. 188° , easily soluble in pyridine and hot alcohol. $[\alpha]_D^{20} = -106.62^\circ$ (pyridine).

(d) *2-Methyl monoacetone d-mannose diethyl mercaptal*.

A thick uncrystallizable syrup.

Trimethyl Mannoses

A 4, 5, 6-trimethyl-2, 3-monoacetone d-mannose diethyl mercaptal, a syrup, is mentioned in the literature (28), but its structure is questionable. Heptamethyl beta-glucosido- α -methyl mannoside, on hydrolysis, results in 2, 3, 6-trimethyl mannose (57, 60), a viscid syrup, showing a rotation of $+6^\circ$ in water at 18° , and $N_D^{20} = 1.4750$. This sugar gives rise to an *anilide*, needles, m.p. $126-7^\circ$ (57), $127-8^\circ$ (60) and to a 2, 3, 6-trimethyl *mannonic acid*, isolated as the *lactone* (45, 57, 60), possessing a melting point of 89° . Methylated glucomannan, on scission, gives rise to 2, 3, 4-trimethyl *mannosaccharic acid* (45).

Tetramethyl Mannoses

Mannose and its oxidative products as well as the alkylated derivatives have been the subject of much study recently, particularly with regard to structural questions (34). Much of the work has been concerned with the tetramethyl derivatives. These are:

(a) 2, 3, 4, 6-Tetramethyl mannose $<1, 5>$ (31, 35, 57, 65, 71).

This sugar is unfermentable by certain organisms (42) and can be transformed to tetramethyl glucose by epimerization (35, 56).

(b) 2, 3, 4, 6-Tetramethyl mannose anilide.

A melting point of $142-3^\circ$ (31, 57, 60) is reported. Its mutarotation has been studied (91).

(c) 2, 3, 4, 6-Tetramethyl mannose phenylhydrazide (31).

(d) 2, 3, 4, 6-Tetramethyl methyl mannoside $<1, 5>$ (55, 65, 107).

The alpha-form has a melting point of $38-40^\circ$, and the beta-form of $36-37^\circ$ (31, 57); $35-60^\circ$ (57, 60). The alpha-form rotates $+43^\circ$ (31, 35, 57) and the beta-form gives -80° (31, 35, 57). The sugar is not fermented by a selected group of organisms (42). The sugar is prepared from the mother sugar (75) or from α -d-methyl mannoside.

(e) 2, 3, 4, 6-Tetramethyl delta-mannonolactone (71, 76).

Obtained from oxidation products of 2, 3, 4, 6-tetramethyl mannose (31, 35, 75). Produces d-trimethoxyglutaric acid on degradation (35) and tetramethyl gluconolactone by epimerization (31). The melting point is $38-40^\circ$ (36).

(f) 2, 3, 5, 6-Tetramethyl gamma-mannonolactone (98).

Prepared from (1) 2, 3, 5, 6-tetramethyl mannofuranose (35); (2) methyl octamethyl galactosido-mannonate (57); (3) mannose diacetone (35); (4) and from d- and l-deltamannonolactone (which indicates a change in the lactone bridge) (90). The lactone is said to have a melting point of $106-7^\circ$ (36), 108° (60), $108-9^\circ$ (57) and a rotation in water of $+63^\circ$ (57), 65° (36).

(g) 2, 3, 5, 6-Tetramethyl mannofuranose (33).

$[\alpha] = +43^\circ$ (equil.) (35).

(h) 2, 3, 5, 6-Tetramethyl methyl mannoside $<1, 4>$ (31, 35).

(i) 2, 3, 5, 6-Tetramethyl mannose phenylhydrazide (57, 60).

This substance possesses a melting point of 167°C .

Alkylated Mannite Derivatives

Syrupy methylated derivatives of mannite are mentioned (136). A 3, 4, 5, 6-tetramethyl mannite monoacetone and a 3, 4, 5, 6-tetramethyl mannite are reported (137).

FRUCTOSE DERIVATIVES

(Iowa State College J. of Science 5:243-250 (1931))

Methylated fructoses described in the literature this last year are:

(a) 1, 3, 4, 6-Tetramethyl γ -fructose (107).

This fructofuranose is reported as the scission product of trimethyl inulin (157), tetraacetyl γ -fructose (121), methylated sucrose (65, 82, 122), methylated melezitose (82), tetramethyl methyl fructofuranoside (65), methylated stachyose (140), and trimethyl methyl fructoside (obtained from trimethyl levan) (70). It distills at $117\text{--}119^\circ$ at 0.05 mm. (122) and the specific rotation is stated to be $[\alpha]_D = +30.7^\circ$ (water) (122) and $+31.3^\circ$ (145). On oxidation, it leads to a gamma-lactol acid (24) which, in turn, forms trimethyl γ -arabonolactone. On attempted osazone formation, a *dimethyl osazone* is formed (145).

(b) *Trimethyl anhydro-fructose* (157).

Said to be obtained from the hydrolysis of trimethyl inulin.

(c) 1, 3, 4, 6-Tetramethyl methyl fructoside (65).

Leads to (a) on removal of the glycoside group. The boiling point is $108\text{--}110^\circ$ at 2 mm. It acquires a pink color with potassium permanganate.

(d) 1, 3, 4, 5-Tetramethyl fructose (normal).

This pyranose form (dl-form has m.p. $95\text{--}96^\circ$ (37, 65) gives trimethyl fructuronic acid (37, 65, 107) on oxidation, and the next step results in the d-lactone of trimethyl arabonic acid. Rotation is $[\alpha]_D -87^\circ$ (145).

(e) *dl-5-monomethoxy fructose* (37, 65).

The result of the condensation of dioxyacetone and α -methoxy- β -oxy-propionaldehyde is this product. It consists of pale yellow crystals, m.p. $80\text{--}85^\circ$, reduces Fehling's, and is hygroscopic. Derivatives are an *osone* (37), and an *osazone*, m.p. 179° .

(f) dl-1, 3, 4, 5-Tetramethyl methyl fructopyranoside.

This compound, melting at 25° , formed from (e). It leads to (d).

(g) Trimethyl γ -fructose.

Secured from methylated sucrose (122).

(h) 3-Methyl fructose (135).

The preparation of this sugar is given by Loder and Lewis (135). They state that it rotates $[\alpha]_D^{20} -54^\circ$ (12 hrs.). The melting point is $122\text{--}3^\circ$. The osazone melts at 164° (65). The compound may be either prepared from the 3-methyl glucose through the common monomethyl enediol (135) or from α -diacetone-3-methyl fructose (m.p. $114\text{--}115^\circ$) (135).

(i) 1, 3, 4-Trimethyl fructose $<2, 5>$ (furanose type).

Trimethyl levan gives this compound (68, 70) on hydrolysis. From it may be prepared trimethyl methyl fructoside, and a dibasic dimethyl lactol acid, identified by the diamide. There is an equilibrium set up between this trimethyl fructose and (j) which indicates a change in the lactone ring (82).

(j) 1, 3, 4-Trimethyl fructose <2, 6>.

Obtained from methylated melezitose (82).

(k) 3, 4, 6-Trimethyl fructose <2, 5>.

This sugar has been recognized in the hydrolytic products of methylated triacetyl anhydro-fructose (144), inulin (142, 146, 156), Yucca dilevan (88), hexamethyl difructose anhydride (145, 155) and pentamethyl h-fructose (145). The boiling point is variously stated to be 90-93°/0.01 mm., 110-114°/0.05 mm., and the rotation as $[\alpha]_D^{20} -38.4^\circ$ (chloroform) (88), and $[\alpha]_D^{15} +25^\circ$ (chloroform) (145). The osazone (113) is stated to have a melting point of 78-80° (144), 79° (88), and 126-7° (146). It is said to give another form (m.p. 77°) in solution (146).

MONO- AND DI-ALKYLATED GLUCOSE DERIVATIVES¹

2-Methyl Glucose

Considerable research work has been done with regard to this sugar because of its importance in structural studies. Oldham and Rutherford (139) have given details of its method of preparation. Paesu (141) has stated that his supposed 4-methyl glucose is probably the 2-methyl compound, and his compounds (28, 29) will be mentioned under this heading. Physical constants of this sugar are:

M.P.	Rotation	Solvent	Reference
157-9°	+66°	Water	61
Syrup			65
157°	64.3°	Water	87
157-8°			139
158°	65.3°	Water	143

It exhibits mutarotation (87, 143), and forms no osazone (65, 139). The osazone, which is reported to have a melting point of 205° (87), is really glucosazone, since the compound loses its methoxyl group when treated with phenylhydrazine (61).

Its derivatives are:

(a) *Monomethyl beta-methyl glucoside* (139).

(b) *Monomethyl-3, 4, 6-tri-p-toluenesulfonyl beta-methyl glucoside* (139).

This substance has a melting point of 168-169° and is sparingly soluble in most solvents.

(c) *6-Iodo-2-methyl-3, 4-di-p-toluenesulfonyl beta-methyl glucoside*.

The melting point is 184-5° (139).

(d) *Monomethyl-3, 4-di-p-toluenesulfonyl beta-methyl glucoside-6-mononitrate*.

Needles, m.p. 157-158° (139).

(e) *Monomethyl-3, 4-di-p-toluenesulfonyl beta-methyl glucoside* (139).

The melting point is 137-140°.

¹ Iowa State College J. of Science 6:33-42 (1931).

(f) *Monomethyl-3, 5, 6-tribenzoyl γ -methyl glucoside*.

(g) *Monomethyl- γ -methyl glucoside*.

This compound has a refractive index of 1.4860.

(h) *3, 4, 6-Triacetyl-2-methyl α -methyl glucoside* (61).

Needles, melting at 120° ; $[\alpha]_D^{20} = +145^\circ$ (chloroform).

(i) *Methyl α -methyl glucoside* (61).

Consists of prisms, m.p. $147-148^\circ$.

(j) *Methyl glucose phenylhydrazone*.

The melting point is reported by different workers as 174.5° (61), 176° (139) and 177° (87).

(k) *Monomethyl benzylidene β -methyl glucoside* (139).

Needles, melting at 170.1° ; $[\alpha]_D = -69.2^\circ$ (chloroform).

(l) *Monomethyl glucose diethyl mercaptal* (87).

(m) *Methyl dibenzyl glucose mercaptal* (143).

2-Thio-ethyl Glucose

Brigl and coworkers (49) are responsible for the description of these compounds. They report a *1, 1-di-ethyl mercaptal-3, 4, 5, 6-tetrabenzoate of 2-thioethyl glucose*, melting $84-85^\circ$, soluble in the usual organic solvents. From this compound it is possible to prepare *1, 1-diethyl mercaptal of 2-thioethyl glucose* and *2-thioethyl-3, 4, 5, 6-tetrabenzoyl- α -l-glucose*. The last-named compound (m.p. $65-66^\circ$, and reducing Fehling's on warming) leads to *2-thioethyl glucose benzamide* of melting point $186-190^\circ$.

3-Methyl Glucose

This alkylated glucose is mentioned but not described in (131) and (139). It yields no lactic acid and no methyl glyoxal osazone (147) under conditions in which glucose yields from 20-55%. It is possible for the 3-methyl glucose to give up its methoxyl group (147). The sugar cannot form a propylene-oxidic glucoside (73). It forms, however, both a gamma and a normal glucoside (73). The alpha-form melts at $160.5-161^\circ$ (143) while the beta-isomer melts lower ($133^\circ-135^\circ$) (65, 143). It exhibits mutarotation, the initial being 74° (20° C.) (135) and the later rotation being 55.3° (19.5 hrs.) (135), -0.54° (54 hrs.) (73).

The derivatives of this important sugar are:

(a) *3-Methyl diacetone glucose* (131, 135).

This compound is used for the preparation of the 3-methyl glucose.

(b) *4-Methyl glucoheptose*.

Prepared from the 3-methyl glucose by the cyano hydrin synthesis.

(c) *Methyl gluconic acid* (135).

Calcium, *brucine* (m.p. $146-8^\circ$), and *cinchonine* salts are reported. The lactone has a final rotation of $[\alpha]_D^{25} +31.8^\circ$ (final).

(d) *Methyl glucose phenylosazone* (96).

The melting point is 164° (65), 178.9° (143) and the rotation is -9° (alcohol) (143).

(e) *3-Methyl glucal* (77).

(f) *Methyl benzylidene β -methyl glucoside* (139).

(g) *Ethylidene derivatives* (63).

(h) *Methyl gamma-methyl glucoside* (73, 114, 131).

(i) *Methyl tetracetyl β -d-glucose* (114).

This melts at $95-96^\circ$; $[\alpha]_D^{20} = -5.2^\circ$ (CHCl_3).

(j) *2, 4, 6-Triacetyl-3-methyl β -methyl d-glucoside* (114).

Melting point is $90-95^\circ$; $[\alpha]_D^{20} -34.8^\circ$ (chloroform).

(k) *2-Acetyl-3-methyl-d-glucose.*

The melting point is said to be 144-144.5°, and the rotation in chloroform at 20° is -40°.

(l) *6-Trityl-2, 4-diacetyl-3-methyl β-methyl d-glucoside.*

This is prepared from (k), and melts at 176.5-177.5°.

(m) *2, 4-Diacetyl-3-methyl β-methyl d-glucoside.*

Results by removing the trityl group from (l). The melting point is 140-142.5° (114).

(n) *2, 4-Diacetyl-3-methyl β-methyl d-glucoside 6-p-toluenesulfonate (m.p. 91.5°).*(o) *2, 4-Diacetyl-3-methyl β-methyl d-glucoside-6-iodohydrin (m.p. 100.5°).*(p) *2, 4-Diacetyl-3-methyl β-methyl d-glucoseenide.*

This compound melts at 76.5°-77.5°.

4-Methyl Glucose

Attempts (138) are being made at this time to prepare this sugar (28, 29, 87, 141, 143) from 2, 3-diacetyl beta-methyl glucoside 6-mononitrate. It has already been reported as being obtained from 2, 3, 6-triacetyl beta-methyl glucoside (64). It has been confused with 2-methyl glucose (28, 29, 141). It is said to be a syrup (143), but one melting point (156-7°) is reported (65).

Derivatives are:

(a) *Methyl-2, 3, 6-tribenzoyl beta-methyl glucoside.*

The rotation is $[\alpha]_D^{21} +58.5^\circ$ (129).

(b) *Methyl beta-methyl d-glucoside (114).*

A syrup.

(c) *Methyl-2, 3, 6-triacetyl β-methyl glucoside (114).*

The melting point is 107-108°, with $[\alpha]_D^{20} -33.5^\circ$ (129).

(d) *Methyl dibenzyl glucose mercaptal.*

M.p. is 98°; $[\alpha]_D -63.9^\circ$ (pyridine) (143).

(e) *Tetracetate of (d).*

This substance melts at 69-70° (143).

(f) *4-Methyl glucose phenylosazone (28).*

The melting point is recorded as being 160° (143), 192-198° (65) and the optical rotation is -15.46° (alc.).

5-Methyl Glucose

Work is in progress on the preparation of this sugar (128). The 5-methyl glucose (141) is said to have a melting point of 143-4° (143), 146° (65) and a final rotation of +59.9° in water. The osazone (65) is claimed to melt at 180° (143) and 185° (65).

6-Methyl Glucose

A slight amount of 6-methyl methyl glucoside is reported as one of the cleavage products of methylated cellulose (96), but it was not obtained pure (128). From this glucoside, an osazone may be prepared, with a melting point said to be 177° (65), 183° (96) and 184.7° (143). The 6-monomethyl glucose (65, 128, 143) has a melting point which apparently varied with the rate of heating (128). It also mutarotates. The 6-methyl monoacetone glucose sintered at about 70°, and melted at 71-72°, and gave a rotation of -6° in chloroform (128). Other derivatives are a *6-methyl gluconic acid* (128), tetraacetyl 6-methyl glucose (m.p. 95-96°), and a

triacetyl 6-methyl β -methyl glucoside. The last-named melts at 104.5°, and —14.5° is its rotation in chloroform (128).

DIMETHYL GLUCOSIDES

Methylated glycogen gave some dimethyl glucose (118) besides the usual hydrolytic products. Dimethyl glucose is mentioned but not described in (141) and (143). Mixtures of dimethyl glucoses have been identified among the alkylated compounds resulting from the scission of methylated cellulose (94, 95, 96, 122) and methylated amylose (157).

2, 3-Dimethyl Glucoses

A large number of 2, 3-dialkylated glucose derivatives have been prepared recently and details of their preparation reported. They are:

(a) *Dimethyl-4, 6-ethylidene beta-methyl glucoside* (63).

The 3-methyl glucose forms this compound, m.p. 108°. $[\alpha]_D^{19}$ —47.75° (chloroform).

(b) *Dimethyl-4, 6-benzylidene beta-methyl glucoside*.

This substance may be prepared from (a). It is reported to have a melting point of 132-135° (139), 133.5-134° (63), and a rotation of —60° at 17° C. It can be prepared, too, from benzylidene beta-methyl glucoside (138).

(c) *Dimethyl methyl glucoside* (138).

A colorless syrup, $N_D = 1.4680$, with $[\alpha]_D = -34.3^\circ$ (water) (138). Can be prepared from (b) (138) or from alpha-methyl glucoside (123).

(d) *Dimethyl beta-methyl glucoside 4, 6-dinitrate* (123).

Melting point 96-99° (138).

(e) *Dimethyl-6-iodo-beta-methyl glucosido-4-nitrate* (123).

(f) *Dimethyl methyl glucoside-4-mononitrate* (123).

(g) *Dimethyl glucose p-toluenesulfonyl chloride* (122).

(h) *6-Iodo-2, 3-dimethyl methyl glucoside* (122).

(i) *Dimethyl glucose*.

This sugar can be prepared from (h) (122), methylated amylose (157), and acetylated cellulose (94, 95, 96, 109). These methods, however, give poor yields.

(j) *Dimethyl-6-iodo-beta-methyl glucoside* (138).

Prisms, m.p. 52-55°, $[\alpha]_D -7.1^\circ$.

(k) *Dimethyl-beta-methyl glucoside 6-nitrate* (138).

A distillable syrup, $[\alpha]_D -27.7^\circ$ (chloroform), prepared from (j) by the use of silver nitrate.

(l) *Dimethyl-4, 6-dibenzenesulfonyl beta-methyl glucoside* (138).

Prisms, m.p. 119-120°; $[\alpha]_D -21^\circ$ (chloroform).

(m) *Dimethyl-4-benzenesulfonyl 6-iodo-beta methyl glucoside* (138).

This derivative possesses a melting point of 72-73° (138).

(n) *Dimethyl-4-benzenesulfonyl beta-methyl glucosido-6-nitrate* (138).

Prisms, melting at 96-97°.

(o) *Dimethyl-4-benzenesulfonyl beta-methyl glucoside*.

Said to melt at 86-7° (138).

2, 6-Dimethyl Glucoses

This sugar is found among the hydrolytic products of methylated amylose (157) and methylated cellulose (94, 95, 96, 109). Derivatives described are the 2, 6-dimethyl-p-toluenesulfonyl chloride (122), 3-iodo-2, 6-

dimethyl methyl glucoside (122), and the 2, 6-dimethyl-3, 4-di-*p*-toluene-sulfonyl beta-methyl glucoside (m.p. 155-157°) (139).

3, 6-Dimethyl Glucose

This dimethyl glucose in the form of the glucoside, is said to be among the dimethyl methyl glucosides secured by the methylation (94, 95, 96) of acetylated cellulose.

TRI- AND TETRA-ALKYLATED GLUCOSE DERIVATIVES

Iowa State College J. of Science 6:43-64 (1931)

TRIMETHYL GLUCOSES

A description of trimethyl glucoses are included in many of the publications which have appeared recently. In some, no description is given (103, 128, 129). A trimethyl glucose is said to have been prepared from the monoacetone derivative of 1, 1-dibenzal mercaptal glucose (87).

2, 3, 4-Trimethyl Glucoses

In the above earlier paper, this trimethyl glucose was described under the heading of 2, 3, 5-trimethyl glucose because a number of workers had wrongly allocated the position of the methoxyl groups to carbons 2, 3 and 4, when they should have been assigned to carbons 2, 3 and 5. Now, however, methods have been evolved for the preparation of the 2, 3, 4-compound, and it is probably correct to assign those positions to the methoxyls in the compounds to be described. Derivatives claimed to be related to the 2, 3, 4-trimethyl glucose are:

(a) 2, 3, 4-Trimethyl glucose (107, 122).

Condensation of this sugar with hydrofluoric acid takes place to the extent of 58 per cent.

(b) *Trimethyl β-methyl glucoside 6-nitrate*.

Secured from (a) as well as from 2, 3-dimethyl beta-methyl glucoside 6-nitrate (138). In turn, it gives rise to

(c) *Trimethyl beta-methyl glucoside*.

This has probably been described in the earlier paper on trimethyl glucoses. Its melting point is said to be 92-93° (122), and 93-94° (138).

(d) *Trimethyl saccharic acid* (51).

(e) 6 (?) *Iodo-trimethyl beta-methyl glucoside* (122).

(f) 6 (?) *Acetyl-trimethyl beta-methyl glucoside* (122).

(g) *i-Xylotrimethoxyglutaric acid*.

The result of the oxidative degradation of 2, 3, 4-trimethyl glucose (51).

(h) *Trimethyl glycuronic acid* (8, 50).

This can be prepared from gum arabic (50), and is possibly formed from mesquite gum (93). In the latter case, it is combined with three molecules of galactose. Said to result, too, from methylated euxanthic acid (86).

(i) *Trimethyl beta-methyl glycuronide* (50).

A non-reducing methyl glucosidic form of methyl glycuronic acid, m.p. 133°.

(j) *Trimethyl 6-triphenylmethyl α-methyl glucoside*.

The product consists of hexagonal plates, m.p. 166-7° (86). It can form

(k) *Trimethyl α-methyl glucoside* (86).

B.p. = 130°/0.1 — 0.2 mm.

(l) *Methyl ester of trimethyl saccharolactone.*

Formed from (k) (86) or from trimethyl glucuronic acid. The crystals are hexagonal plates, m.p. 106° (86), 107° (51) with $[\alpha]_D^{22} +104.3^{\circ}$ (alc.) (51). See (86) for solubilities.

(m) *Methyl dimethoxysuccinic acid.*

The product results from the oxidation of 2, 3, 4-trimethyl glucuronic acid.

2, 3, 5-Trimethyl Glucose

This sugar results from the scission with acids of trimethyl l-glucosan (16).

3, 4, 6-Trimethyl Glucose

This is reported as being the product from methylated alpha-glucosan (154). Mentioned only in (122).

4, 5, 6-Trimethyl Glucoses

This sugar has been mentioned in a number of papers (28, 29). A compound, to which this structure was assigned, has been shown to be a 4-methyl glucose (143). Likewise the osazone was shown to have only one methoxy group (141). The corrections which have been made with regard to the loss of the methoxyls brings up the question as to whether tri- and tetramethylated derivatives could lose a methoxy on osazone formation (141).

2, 4, 6-Trimethyl Glucose

The melting point is given as $121-123^{\circ}$ (122). A 2, 4, 6 (?) -trimethyl-p-toluenesulfonyl beta-methyl glucoside (m.p. $104-105^{\circ}$) is described (122).

2, 3, 6-Trimethyl Glucoses

This sugar is mentioned in 53, 55, 107, 117, 119. Its derivatives are numerous:

(a) *Trimethyl glucose.*

Trimethyl glucal can be used (77) for its preparation. It condenses to the extent of 59 per cent with hydrofluoric acid (115). The melting point is variously stated to be $110-112^{\circ}$ (118), 112° (95), 114° (123) and 121° (120). The rotation is 70.9° in water (120). The trimethyl glucose may also be prepared from octamethyl maltose (24), methylated amylose (157) and trimethyl beta-methyl glucoside (66, 67, 94, 95, 96, 109, 110 and 111).

(b) *Trimethyl-5-p-toluenesulfonyl γ -methyl glucoside* (122).

This does not react with sodium iodide (138).

(c) *Trimethyl glucono- γ -lactone* (120).

(d) *Trimethyl gamma-methyl glucoside* (117).

The boiling point is $180^{\circ}/0.05$ mm., with $[\alpha]_D = -26.3^{\circ}$ (water) (120).

(e) *Trimethyl glucono- δ -lactone* (120).

(f) *Trimethyl glucose anhydride* (107, 123).

A mobile oil of a low boiling point (55).

(g) *Trimethyl methyl glucoside-4-mononitrate.*

The refractive index was found to be 1.4595 (123).

(h) *Trimethyl beta-methyl glucoside* (104).

This glucoside results from (g) (123), methylated glycogen (110, 111, 118), and trimethyl cellulose (66, 67, 94, 95, 96, 98, 109). The glucoside boils at $130-140^{\circ}$ at 0.2 mm., and has a refractive index of 1.4595 (123).

(i) *Trimethyl-4-benzenesulfonyl beta-methyl glucoside.*

This melts at 83-84°; small cubes soluble in all solvents except water and petroleic ethers (138).

(j) *Trimethyl gluconic acid (117).*

TETRAMETHYL GLUCOSES

2, 3, 4, 5-Tetramethyl Glucoses

Haworth (107) mentions the 2, 3, 4, 5-tetramethyl gluconic acid and the 2, 3, 4, 5-tetramethyl saccharic acid.

2, 3, 4, 5-Tetramethyl Glucoses

Usually, when the positions of the methoxyl groups are not specified, references to "tetramethyl glucose" refer to this sugar (55, 78, 94, 95, 96, 122, 141). It is one of the most important alkylated sugars in the study of structural carbohydrate chemistry. This tetramethyl glucose, and its compounds, has been described in detail in the earlier paper. Recent publications give the following additional information.

(a) *2, 3, 4, 6-Tetramethyl glucose (78).*

The product results from the splitting of methylated melezitose (82), glycogen (110, 111), amylose (157), cellan (115), and from the hydrolysis of the glycoside group of the alpha and beta glucosides (109, 120, 128, 131). The compound is not oxidized by oxygen in the presence of iron pyrophosphate (52), and condenses (68 per cent) with hydrofluoric acid (115). It is possible, by formation of a common ene-diol, to pass over from the tetramethyl glucose to the corresponding tetramethyl mannose (135). The sugar is crystalline (120) and dextro-rotatory: the melting point being given as 87° (95), 88-89° (110, 111), 89° (109), and 88-90° (128); the rotation as +83° (equil.) (110, 111), 83.5° (109) and 94° (50 per cent sulfuric acid) (103). A simplified procedure for its preparation has been given by West and Holden (152).

(b) *Tetramethyl beta-ethyl glucoside (65, 107).*(c) *Tetramethyl beta-methyl glucoside (98, 107).*

Can be secured from methyl glucoside (131), and methylated cellulose (98, 109, 157). Leads to 2, 3, 4, 6-tetramethyl glucose (109, 131). The melting point is 40-41° (103, 105), the boiling point is 80-85°/0.1 mm., and the rotation -18° in water (65, 103).

(d) *Tetramethyl gluconic acid phenylhydrazide.*

M.p. 115°; $[\alpha]_D = +42.18^\circ$ (alc.).

(e) *Tetramethyl glucose anilide.*

M.p. 137-138° (128).

(f) *Tetramethyl alpha-methyl glucoside (98, 107).*

The sources of this sugar are 2, 3, 6-trimethyl alpha-methyl glucoside (120), methylated cellulose (98, 109) and the parent tetramethyl glucose (120). The rotation is given as +101° (128).

(g) *Tetramethyl delta-gluconolactone (107).*

Oxidizes to xylotrimethoxyglutaric acid.

(h) *Tetramethyl gluconic acid (107).*(i) *Tetramethyl glucose-1-chlorohydrin (104).*

Used with trimethyl beta-methyl glucoside to synthesize heptamethyl beta-methyl cellobioside.

(j) *Tetramethyl 2-keto-gluconic acid amide (107).*

2, 3, 5, 6-Tetramethyl Glucoses

This tetramethyl glucose (73, 107, 117, 119, 131) may be prepared (112) from either the alpha-methyl glucofuranoside or the alpha-ethyl glucofuranoside. The compound is a syrup (120), distilling at 130°/0.01 mm., 130°/0.15 mm. (112, 120), and with the specific rotation $[\alpha]_D = -15.4^\circ$ (benzene) (120), and -7.6° (112).

It forms:

(a) Tetramethyl α -methyl glucofuranoside (107).

Can be secured from the methylation of 3-methyl d-glucose or d-glucose (73). The optical rotation is $+104^\circ$ (65) and $+106.5^\circ$ (112). It distills at 94° with 0.04 mm. pressure (112).

(b) Tetramethyl gluconic acid (107, 120).

(c) Tetramethyl gluconic acid barium salt (73).

$[\alpha]_D^{22} = +33.2^\circ$ (water).

(d) Tetramethyl gluconic acid phenylhydrazide (73, 112).

M.p. = 135° (112); 135.8° (corr.) (73).

(e) Tetramethyl gamma-methyl glucoside.

Distills at 115°/0.35 mm.; $[\alpha]_D^{15} = -16.0^\circ$ (water) (120). May be formed from 2, 3, 6-trimethyl gamma-methyl glucoside (117, 120).

(f) Tetramethyl glucono- γ -lactone (112, 120).

Methylated manninotronic acid gives this lactone (140). A melting point of 26° (112) is reported.

(g) Tetramethyl beta-ethyl glucofuranoside (112).

ALKYL DERIVATIVES OF DI- AND POLY-SACCHARIDES

Iowa State College J. of Science (1933)

Disaccharides

Octamethyl cellobiose has been mentioned in 125, 150, and 156. Further work (155) has been done on the difructose anhydride which gives a hexamethyl derivative and forms 3, 4, 6-trimethyl fructofuranose on hydrolysis.

Trisaccharides

Hess and coworkers have continued their work on the interesting hendecamethyl cellotriose which has a melting point of 116-117° (116, 125, 149, 150, 156). Onuki (140) has reported a manninotriose from stachyose, which can be oxidized to a manninotronic acid. The acid forms a *undecamethyl manninotronic acid*, $[\alpha]_D^{22} = 1.4597$, which leads to 2, 3, 5, 6-tetramethyl d-gluconic acid lactone and 2, 3, 4, 6-tetramethyl galactose on hydrolysis.

Tetrasaccharides

Tetradecamethyl cellotetraose (m.p. 134-138°) is secured along with the methylated cellotriose mentioned above (116, 125, 149, 150). It is prepared from the water soluble dextrin from cellulose. Tetradecamethyl stachyose is a colorless syrup (140).

Polysaccharides

An intensive study has been made by Bell (94, 95, 96) on the cleavage products of various wood celluloses. He found that wood contains a resistant portion which does not methylate easily. Kruger (126) has reviewed the recent experimental and theoretical advances in the chemistry of cellu-

lose and concludes his paper with 77 references. The methylation of cellulose has been discussed (109, 159). Methylated cellulose leads to 2, 3, 6-trimethyl glucose in the first stage of hydrolysis (157). The same fact is true of methylated amylose (157).

The problems in connection with the methylation of glycogen have been further studied (111). Irvine (157) has published on the hydrolysis of trimethyl inulin.

Miscellaneous

Trimethyl-N-methyl guanosine hydrochloride is a gum, decomposing at 98°, and giving trimethyl ribose (132) on hydrolysis.

GENERAL

Prof. E. O. von Lippmann has continued his semi-annual reviews in the field of pure carbohydrate chemistry (21, 27, 43), and emphasizes the importance of alkylated derivatives in structural studies. Pringsheim (83) has presented an outline of the present-day sugar chemistry and Freudenberg (55) has written on certain aspects of the chemistry of cellulose and other polysaccharides. The methylation of glucose with dimethyl sulfate and sodium hydroxide at a temperature of 30° in a solution kept alkaline to bromothymol blue and acid to phenolphthalein resulted in a product which definitely gave a positive response to several common tests for an active or gamma-sugar (15).

Theories of structure of carbohydrates have been summarized in a paper by Csürös (99). Ohle (81) has likewise presented an extensive review of the modern structural concepts. Bibliographies on methylated carbohydrates may be found in the publications of Bridel (48), Irvine (4), Hudson and Pringsheim (39), and in the recent excellent volumes of Abderhalden (VIII), Vogel and Georg (151), and of Haworth (The Constitution of Sugars. London: Edward Arnold and Co., (1929). The last-mentioned has been recently translated into German. Berger (17) claims that a dimethyl hexose (osazone, m.p. 206-8°), not present as a glucoside, was found in *Erysimum crepidifolium*.

Methods for determining methoxyl groups in carbohydrates have been worked out (22, 105). Several other papers mentioned methylated sugars only in a general way (44, 85). A trimethyl monoacetone idose is described by Ohle and Vargha (46) and a dl-5-methoxy- α -acrose by Hiersant and Linnell (65).

NATURAL ALKYLATED CARBOHYDRATES OF REBATEABLE STRUCTURE

These will not be described in this paper because of their questionable character, but detailed description will be found in the following references:

Cymarose: 10, 20, 25, 26, 40 and 124.

Digitalose and derivatives (From digitalin and oleandrin): 1, 3, 5, 9, 12.

Digitoxose methyl ether: 10.

Sarmentose: 124.

Strophantibiose methyl ether: 2, 6, 7, 11, and 148.

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OXYGEN ABSORPTION IN SOILS¹

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The production of carbon dioxide in soils has long been regarded as a measure of microbiological activity and since the fertility or crop-producing power of a soil is dependent to a great extent upon microbiological action, carbon dioxide production has come to be considered an index of the fertility of the soil. Russell (2), however, regarded the rate of oxygen absorption in soils a more reliable measure of fertility than the rate of production of carbon dioxide. In his work, a simple manometric method was employed for determining the rate of oxygen absorption.

Smith and Brown (3) adapted the Barcroft (1) differential manometer for measuring oxygen consumption in soils. This manometer seems to possess many advantages over the manometer used by Russell. A critical investigation of the method has been made and the purpose of this paper is to present the details of the method and some results secured.

EXPERIMENTAL

PROCEDURE

The apparatus used to measure oxygen absorption (Fig. 3, and Plate I), consisted of a manometer, each arm of which was attached to a 500 cc. flask and could be opened to the atmosphere. A side arm was sealed into the right flask of one manometer for sampling the air for analysis. Inside each flask, a vertical tube 25 mm. in diameter and 40 mm. high, was sealed to the bottom to hold the sample of soil. Soil placed in the small cup of the right flask absorbed oxygen and gave off carbon dioxide. The carbon dioxide was absorbed in potassium hydroxide, placed in the large flask, and the amount of oxygen absorbed by the soil was measured by the change in the manometer reading.

The method suggested by Stephenson (4) was adapted for calibrating the manometers. Ten grams of air-dry soils were placed in the small cup of the right flask and the moisture content adjusted by adding distilled water. Ten cubic centimeters of a 35 per cent solution of potassium hydroxide were placed in the bottom of the right and left flasks. The flasks were then attached to the arms of the manometer and placed in the constant temperature water bath, with the manometer outside the water. The calibrating pipette was placed in a deep museum jar with circulating water from the constant temperature bath. The room temperature was about the same as that of the water in the constant temperature bath, and no difficulty was experienced in maintaining the temperature in the glass jar the same as that of the water bath. A standard, one cubic centimeter pipette, calibrated in hundredths, was connected at one end to the right arm of the manometer by means of a capillary glass tube and rubber tubing and at the other end by rubber tubing, to a long glass tube for leveling. The leveling tube and pipette were filled to a convenient level with kerosene

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colored with Sudan III. After the apparatus had remained in the water bath approximately 30 minutes to attain the temperature of the bath, the left flask was closed to the atmosphere and the oil leveled by raising or lowering the leveling tube. The right flask was then closed and a reading of the manometer level and liquid in the pipette made after which the flask was opened and the leveling tube lowered, sucking air out of the flask; it was then closed and the apparatus shaken in the water bath for 30 seconds; the liquid in the pipette was leveled and a reading made. The readings were then checked by returning the oil to the position in the first reading, which duplicated the first readings if no leaks or temperature change had occurred. The difference between the first and second readings of the pipette in cubic centimeters, reduced to standard conditions of temperature and pressure, divided by the difference between the first and second readings of the right and left arms of the manometer, multiplied by 1000, gives the flask constant, which when multiplied by the rise in height of the manometer liquid, gives the cubic millimeters of oxygen absorbed at 0° and 760 mm.

The procedure followed in these experiments consisted in placing 10 gms. of soil in the small cup of the right flask, adjusting the moisture content, placing 10 cc. of a 35 per cent solution of potassium hydroxide in the bottom of the right and left flasks and then placing the flasks in the water of a constant temperature water bath which could be regulated to $\pm 0.005^\circ\text{C}$. The moisture content was adjusted to 30 per cent unless otherwise stated and the temperature of the water bath was regulated to 30°C . unless otherwise stated. The soils used were Carrington loam and Tama silt loam.

RESULTS

The influence of aeration on the rate of oxygen absorption in Carrington loam was determined in the first experiment. Ten grams of air-dry soil were placed in the small cup of the right flask and the moisture content adjusted to 25 per cent. The flasks of manometer I were aerated 10 minutes with CO_2 -free air before being attached to the manometer. The flasks of manometer II were not aerated. The constant temperature bath was regulated to 25°C . Readings were made at intervals during 28 hours and the oxygen absorbed calculated. The results are shown in figure 1. Curve I, figure 1, represents the flasks which were not aerated and curve II represents the results from the soil which was aerated.

The initial rate of oxygen absorption was greater in the unaerated flask than in the flask aerated with CO_2 -free air.

In another experiment with Carrington loam, the cocks of one manometer were opened at intervals after making the readings, allowing the manometer liquid to return to zero and the flasks to fill with air. The results are shown in curves III and IV, figure 1.

The results shown by curve IV, figure 1, represent the flasks opened to the atmosphere at intervals, and indicate no effect on the rate of oxygen absorption. The two curves are approximately parallel but of slightly different magnitude.

The influence of dextrose on the rate of oxygen absorption was studied in two experiments, one with Carrington loam and the other with Tama silt loam. Two manometers were used in each experiment. In the experiment with Carrington loam, the manometer liquid was drawn over into the flasks during the night. The results are shown in curve V, figure 1. In the experiment with Tama silt loam, the manometer cocks were left open

during the first 15 hours, then closed and readings taken during the next 9 hours. The results are shown in curve VI, figure 1.

These two curves are approximately parallel, the only difference being in the magnitude of the absorption at any given time.

The rate of oxygen absorption in untreated Tama silt loam was determined over a period of 114 hours. Two manometers were used in this

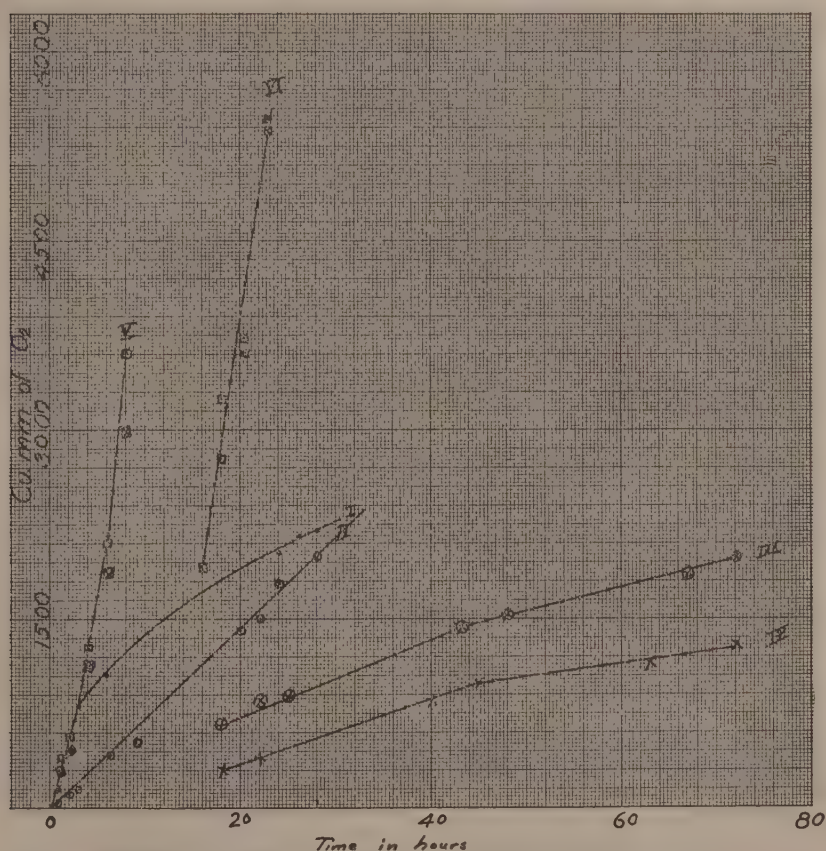


Fig. 1. Influence of aeration and dextrose on oxygen absorption in Tama silt loam

experiment. Readings were made at intervals and the amount of oxygen absorbed calculated. The results are shown in curve I, figure 2. The duplicate tests checked closely; there is indicated a gradual slowing up of the respiration for 48 hours, after this period of time there followed a more marked decrease in the rate of respiration.

The rate of oxygen absorption by soil in an atmosphere of oxygen and in air was determined in another experiment. Tama silt loam was used as in the preceding experiment, except that the air of the flasks of one apparatus was replaced with approximately pure oxygen. Readings were made over a period of 124 hours. The results secured are shown in curves II and III, figure 2.

The amount of oxygen absorbed by the soil in oxygen is shown in

curve III and the amount absorbed by the soil in air is shown in curve II, figure 2. During the first 92 hours of the experiment, oxygen absorption by soil in air was much greater than in an atmosphere of almost pure oxygen. However, the final rate of absorption was greater in oxygen than in air. The manometer liquid in the apparatus with the flasks filled with oxygen registered a decrease at several intervals. No satisfactory explanation for the fall in the manometer liquid can be offered at this

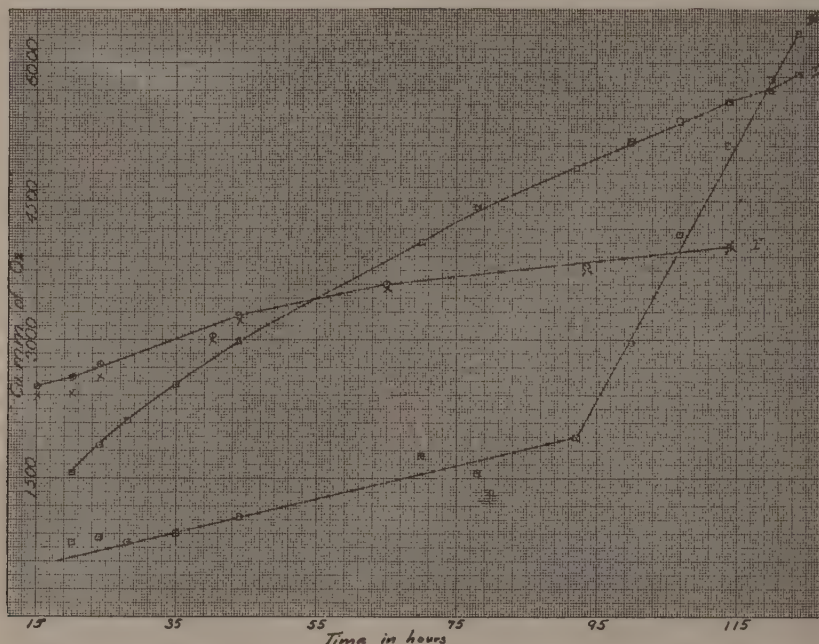


Fig. 2. Rate of oxygen absorption by soil in air and in oxygen

time. It could have been caused by a leak in the right cock of the manometer. The cocks were kept well greased and when tested for leaks were found to be air tight. That this fall in the manometer liquid was not caused by leaks around the cocks of the manometer, is shown by the results of other experiments where the liquid did not stop at zero but continued to rise in the left arm of the manometer. The production of a gas other than carbon dioxide in the right flask or a change in temperature in this flask would also cause the liquid in the manometer to fall. Anaerobic conditions would be necessary for the production of a gas which would cause this pressure and it is unlikely that anaerobic conditions could exist; however, this point was studied further in another experiment.

Two manometers were set up using Tama silt loam which had been passed through the 20-mesh sieve in one manometer and Tama silt loam which had been ground to pass the 100-mesh sieve in the other manometer. The volume occupied by 10 gms. of the soil is increased by grinding but the porosity may be decreased if the sample is packed into the same volume as the unground soil. The individual pores in the ground soil are, at least, smaller than in the unground soil; are more nearly filled with the added water and thus conditions might be secured which more nearly approach

anaerobic than those in the unground soil. The rate of absorption of oxygen by the 20-mesh soil is given in curve I, figure 4, and the rate of absorption by the 100-mesh soil in curve II, figure 4.

The initial rate of oxygen absorption was slightly lower in the finely ground soil than in the 20-mesh soil but the final rate was increased considerably. While these results do not prove aerobic conditions of the soil, they indicate that the lowering of the manometer liquid was not caused by anaerobic gas production. In order to test this point further, the following experiment was carried out.

Ten grams of air-dry soil were placed in the bottom of the 500 cc. Erlenmeyer flasks and the KOH was placed in the small cups. More sur-

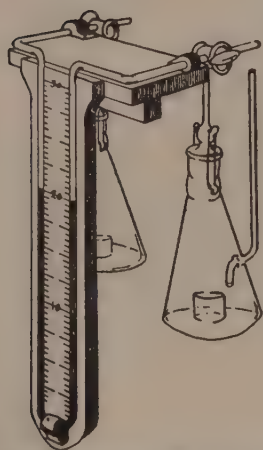


Fig. 3. Modified Barcroft differential manometer

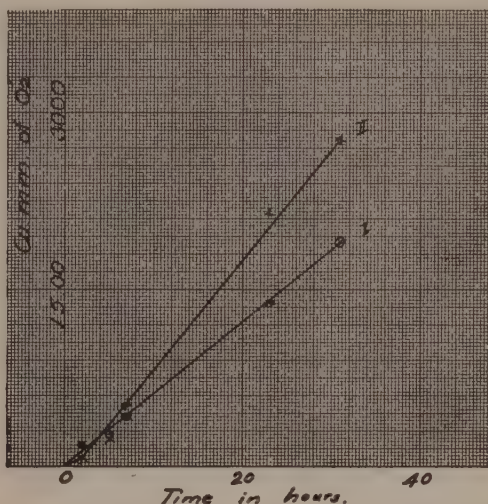


Fig. 4. Influence of grinding on rate of oxygen absorption by soil.

face of the soil was thus exposed to the atmosphere of the flask. The flask of one apparatus was filled with approximately pure oxygen and the flask of the other apparatus was filled with air. Readings were made over a period of 105 hours. The results are recorded as millimeters of manometer liquid and are shown in figure 5. Curve I represents the soil in air and curve II represents the soil in an atmosphere of oxygen.

These results indicate the evolution of a gas in both apparatus. A high initial pressure was developed in the flask with oxygen but normal oxygen absorption apparently occurred in the flask filled with air until after 60 hours when a pressure was developed. It is also evident that the soil in these flasks was not under anaerobic conditions, nor was the fluctuation in the manometer liquid caused by leaks around the manometer cocks as these would have been evidenced in the pressure in the flask filled with oxygen.

When the soil was placed in the small cups, it is probable that sufficient heat was developed in the soil to account for the fluctuations in the manometer liquid but certainly this development of heat did not occur when the soil was spread over the bottom of the 500 cc. flask. The flasks

were set up with soil in the small cups in the usual way, fitted with a Beckman thermometer and placed in the constant temperature water bath. The temperature readings are shown in table 1.

The results in the table show a slight variation in temperature of the soil during 16 hours, the greatest variation being 0.039°C . Assuming that

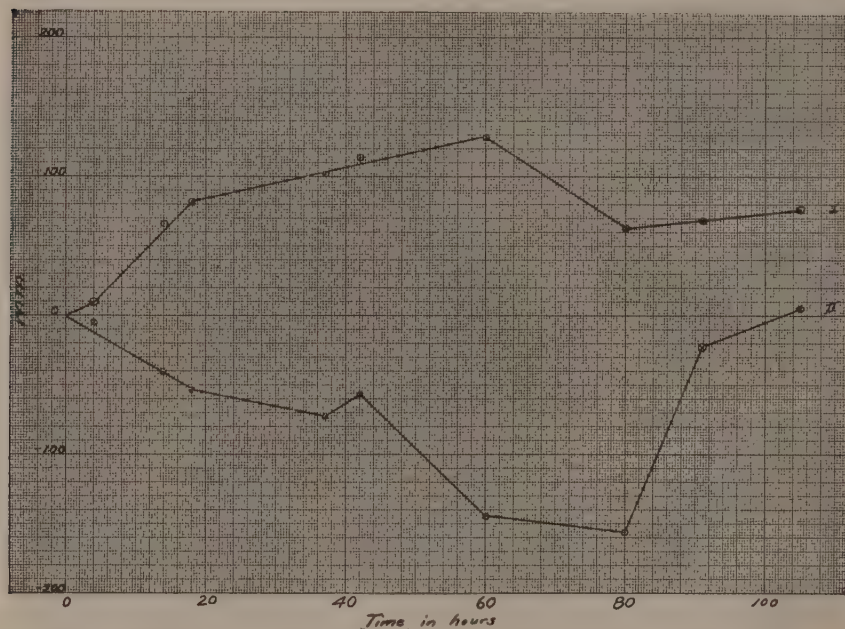


Fig. 5. Fluctuations in height of manometer liquid; I, air; II, oxygen.

the entire content of the flask was influenced by this increase in temperature, it would account for less than 2 mm. pressure in the manometer liquid.

The fluctuation in pressure in the manometer flasks appeared unusual since the method has been used by many investigators working along similar lines and no record of such behavior had been found in the literature. Russell (2) studied several factors influencing oxygen absorption in soils, such as moisture content of soil, temperature and fertility of the soil. In one case he recorded "a slight evolution of gas" brought about by an excess of moisture. Several tests were carried out in this work with various

TABLE 1. *Temperature changes in soil within flask*

Time	Reading of Beckman thermometer	Change in temp. degrees C.
0	4.323	0
1:30	4.323	0
2:30	4.323	0
7:00	4.357	0.034
16:00	4.362	0.039
18:30	4.320	-0.003
21:00	4.330	0.007
23:00	4.320	-0.003

moisture contents of soil and it was observed that this pressure (evolution

of gas) occurred at low moisture content as well as at high moisture content. The temperature of the water bath was even and constant. Therefore, the fluctuations were not caused by temperature differences outside the flasks. The heat of wetting of the soil was determined and sufficient time was allowed for the flasks to come to the same temperature as that of the bath before closing the cocks. Even though heat might have been developed in the small cups containing the soil, it is quite unlikely that this was the cause of the fluctuations in the manometer liquid as two small samples of the same soil, treated as nearly as possible alike, might be expected to act in the same way. The pressure, then, was apparently due to the evolution of a gas. Samples of air from the flasks taken for analysis showed a decrease in oxygen, no carbon dioxide and no combustible gases, such as hydrogen or methane. It is unlikely also, that nitrogen gas was evolved, since the gas was evolved in an atmosphere of nearly pure oxygen and the nitrate content of the soil was low.

SUMMARY AND CONCLUSIONS

The results secured with the manometers do not represent oxygen absorption alone, but a resultant of several processes, the simplest might be oxygen absorption and the evolution of some non-combustible gas, insoluble in potassium hydroxide. The pressure produced in the flasks was not produced entirely by a difference in temperature either outside or inside the flask. No combustible gases (hydrogen or methane) were found. The pressure was produced at low moisture content of soil and at high concentration of oxygen. The nitrate content of the soil was low and it seems unlikely that nitrogen gas was evolved through denitrification.

It is possible but seems unlikely, that under rapid oxidation, such as would take place with dextrose in soil in an atmosphere of oxygen, higher temperatures were reached in the experiments where a pressure was produced than were measured by the Beckman thermometer. This possibility is being studied further.

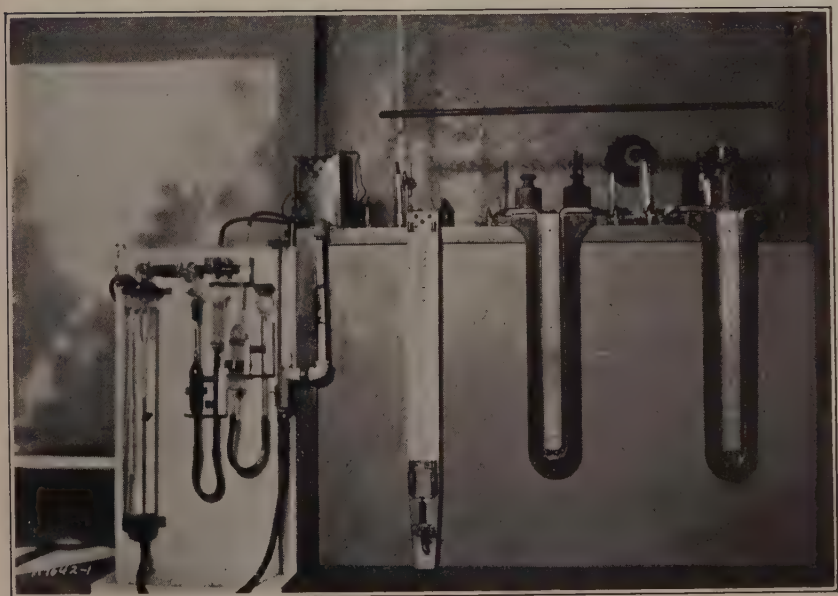
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Plate I

Constant temperature waterbath and manometers.

Plate I



ELECTRON TUBE POTENTIOMETER FOR THE DETERMINATION OF REDOX POTENTIALS¹

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Relatively little is known regarding redox equilibria in bacterial cultures, and studies correlating electrode potentials developed by micro-organisms with physiological behavior offer a promising field to the biologist.

Since the electron-tube potentiometer for the measurement of redox potentials is in a state of development, and the apparatus and its use embody certain conceptions and terms unfamiliar to many biologists, it was felt desirable to relate our experiences and give the details of the construction of several electron tube potentiometers with which we have worked on bacterial cultures during the past two years.

Living cells have the ability to influence the reversible redox potentials of suitable media. This change in potential can be determined by the use of electrodes of the noble metals such as platinum or gold. It is necessary in making these potential determinations to avoid polarization of the biological system, and to do this the use of an electron tube potentiometer is recommended. Thus if we can accurately determine the time-potential graph of a bacterial culture, such a graph should express a definite relationship to the reversible biological oxido-reduction processes taking place within the culture.

Courtines and Geloso (1930), Plotz and Geloso (1930), Elema (1932) working in Kluver's laboratory and Allyn and Baldwin (1932) have used the electron tube in the determination of redox potentials. The reader is referred especially to the monograph of Elema for a thorough discussion. This investigator, also used the apparatus with a glass electrode for the determination of pH values. Allyn and Baldwin do not mention the type of tube employed in their work; Plotz and Geloso (1930) used a tube permitting measurement of one millivolt with a grid current of 10^{-15} amperes. Their circuit is substantially that used by Allyn and Baldwin. Elema (1932) appreciated fully the desirable characteristics of a tube to be used in making redox potential readings and pH determinations with a glass electrode. He used the Philips tube 4060, for which the maximum grid current is advertised as of the order of 10^{-14} amperes, though the grid-plate transconductance of this tube is quite low and the cathode current consumption is high—1.0 ampere.

DuBois (1930) used an electron tube potentiometer which was operated successfully with glass electrodes having a resistance as high as 100,000 megohms. He employed the common '22 type screen grid radio tube. The circuit was a modification of that of Stadie (1929) which was less complicated and in which drift had been eliminated.

Fosbinder (1930) describes an electron tube potentiometer for use

¹ Supported by the Industrial Research and Rockefeller Fluid Research Funds of Iowa State College.

with high resistance systems. He likewise used the screen grid '22 type tube.

DISCUSSION OF ELECTRON TUBES AND THEIR CHARACTERISTICS

It is desirable that the biologist who plans to construct an electron tube potentiometer refer to one of the standard radio manuals in order to familiarize himself with certain basic definitions.

The electron tube consists of an evacuated glass bulb within which are placed a filament (cathode), a grid and a plate (anode). The heated filament emits electrons which pass to the positively charged plate. The plate current depends upon plate voltage, temperature of filament, resistance of external and internal plate circuits, the grid resistance and the grid potential. Upon the grid is generally imposed a negative charge. Any fluctuation of the grid voltage is reflected on the plate current, any decrease of the negative charge will result in a greater flow of the electrons from the filament to the plate, whereas, if the grid becomes more negative the flow of electrons will be repulsed. The correct voltages to be applied to the electrodes are determined by the characteristics of the tube. The unknown potential of the biological system is placed on the grid. It is more accurate not actually to measure the current change on the plate, but to compensate potentiometrically for this change, thus using the tube as a zero instrument. The biological system does not supply any current to operate the galvanometer; the plate current and a feed-back from the filament current serve this purpose. The grid current is extremely small depending upon the characteristics of the tube. For very accurate work the General Electric Company has placed on the market their FP-54 tube. A current of 1×10^{-15} ampere (equivalent to approximately 63 electrons per second) may be measured accurately with this tube.

A relationship exists between grid voltage and filament voltage where the grid current is zero and polarization of the biological cell is reduced to a minimum during measurement of the voltage. This grid potential at which the grid current is zero is called the floating grid potential and is the potential assumed by the grid on open circuit in an ideal tube. In our work no attempt was made to work the tube at its floating grid potential, since the grid current of our circuits was sufficiently low to preclude polarization disturbance. It is important to shield the grid and its parts carefully.

The electron tubes in the potentiometric apparatus to be described, function as voltage amplifiers to produce marked current change in the plate (anode) circuit induced by very small voltage change on the grid by the reversible biological oxidation-reduction systems. Figure 1A, illustrates this current magnification induced by grid voltage change. The high ratio of change of plate current to grid voltage change at low grid voltage is apparent.

The essential consideration in constructing an electron tube potentiometer is the choice of a suitable tube with proper circuit. We have used a number of tubes all obtainable from radio dealers. The very sensitive FP-54 tube of the General Electric Company is not considered here because of its cost.

The characteristics of a tube may be defined as the distinguishing electrical features and values which serve to identify the tube. Characteristics may be plotted (see figure 1A) or tabulated.

It is desirable to choose a tube having a large mutual conductance

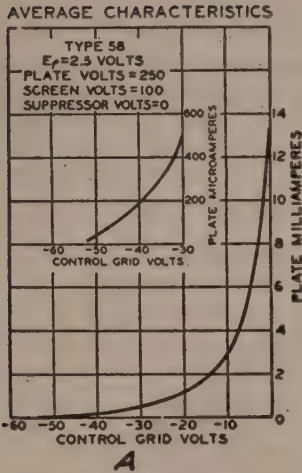


Fig. 1A. Graph showing change in plate current with change in grid potential. (Courtesy Radio Corp. of America)

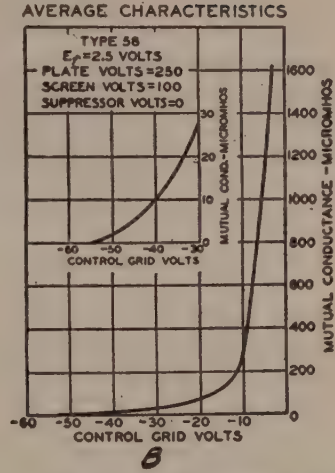


Fig. 1B. Graph showing high mutual conductance at low negative grid bias. (Courtesy Radio Corp. of America)

(grid-plate transconductance) defined as the ratio of change in the plate current to (and produced by) a small change in the grid voltage under conditions of constant plate voltage. Tubes of the type '12A, '22, '24A, '32, 58 and '99 used in the apparatus to be described have large mutual conductances. Harrison (1930) pointed out that if a high resistance of the order of 10^8 ohms is placed in the grid circuit, a considerable change occurs in this characteristic of the tube since the filament-grid resistance of the ordinary tube is of the order of magnitude of 10^8 ohms.

The voltage sensitivity is the change in plate current with change in grid voltage under conditions of operation, that is, all resistances in the circuit are small in comparison with those in the external grid and in the plate circuits. Since the resistance of the culture is low, the maximum sensitivity of the tube is substantially equal to the mutual conductance. The grid current of the modern tube at the grid potential employed is very low and general experience indicates too low to cause appreciable polarization.

At a certain low value of the imposed grid potential change, deflection of the galvanometer needle due to zero drift becomes of a magnitude comparable to that caused by the imposed grid voltage change. It is obvious that drift from zero galvanometer reading while the electron tube potentiometer is in use is annoying. It is to be noted that this drift may be caused by the fluctuation of electronic emission from the filament when the drift is uniform and in one direction or to body capacity when the drift is violent and in both directions. Shielding of the set and the use of batteries in good condition reduces zero drift to a minimum. The grid lead should be kept short.

The amplification factor, or μ is the ratio between a small plate voltage change and grid voltage change, both producing the same plate current change. Thus, if a grid change of 5 volts and a plate change of 10 volts, both independently produce the same plate current change, the amplifica-

tion factor is $\frac{10}{5} = 2$. A tube with an amplification factor of four permits twice the plate voltage variation with a resulting plate current change of the same amperage as the former tube. The amplification factor is an expression of the ability of a tube to amplify voltages. A tube having a high amplification factor is commonly referred to as a 'high μ ' tube. In the present application of the electron tube, the plate current is maintained constant. The amplification factor is related to the mutual conductance according to the equation:

$$\text{mutual conductance} = \frac{\text{amplification factor}}{\text{plate to filament resistance}}$$

It is desirable to choose a tube which operates on a low current consumption unless it is to be operated from a lighting circuit. Tubes of the '99 and '32 type operate on 0.06 ampere, whereas, the '35 tube consumes 1.75 amperes which would be a heavy drain on a storage or dry battery, and would lead easily to current fluctuation on the filament.

Filament current and grid current fluctuations were reduced to a minimum by using part of the filament current to pass through the galvanometer in opposition to the plate current. In the case of tubes 58 and 57 a battery furnishing 1.5 volts was supplied since these tubes do not employ a direct filament current. Introducing the opposed current by either method has the advantage of permitting the galvanometer to be operated as a zero instrument.

The electrodes of an electron tube act as plates of a condenser, thus forming an electrostatic system. The condenser action (capacitance) between the grid and the plate is of greater importance than that between the cathode and plate or that between cathode and grid. The most effective method of avoiding influence of grid-to-plate capacitance is the use of a fourth element or electrode called the screen, placed between the plate and the grid. The screen voltage determines largely the electron-flow, and the small changes of plate voltage have only a negligible effect on plate current and this is desirable from the standpoint of stability of operation. The screen does not prevent the flow of electrons to the plate but does act as an electrostatic shield between the grid and plate and reduces capacitance of the grid-plate.

In the pentode electron tube (type 57 and 58) a fifth electrode called a suppressor is added to prevent escape of electrons from the plate, which occurs if electrons moving at a high rate of speed strike the plate and dislodge electrons. This escape of electrons is termed secondary emission. In the case of screen grid tubes of the tetrode type the presence of the screen in close proximity to the plate strongly attracts these electrons, lowering the plate current and limiting the permissible plate swing. In our use of the screen grid in tube 58, it has been tied into the plate circuit and carries a charge less positive than the plate, thereby retarding the flight of vagrant electrons from the plate.

Figure 1B shows the mutual conductance of the 58 tube at different grid voltages, reaching a maximum characteristic at the lower voltages. We have used generally, -1.5 volts on the grid electrode through a resistance. Figure 2 shows the effect of plate voltage change on plate current change at different grid potentials.

An electron tube is referred to as a triode, tetrode or pentode depending upon the number of elements.

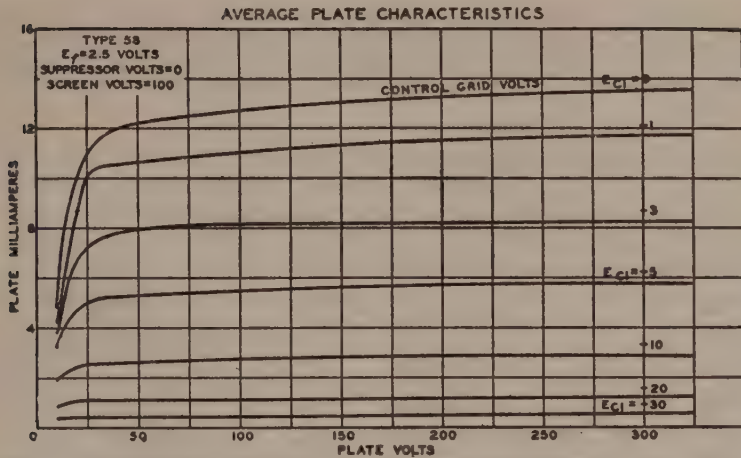


Fig. 2. Graph showing change in plate current with change in plate voltage. (Courtesy Radio Corp. of America)

Filament or heater current. Either dry cells, air-cell battery or storage batteries may be used to furnish current to a filament. The use of discarded automobile batteries which have only one cell useless is recommended for economy. Tube '32 requires 2.0 volts and the '99 operates on 4 volts with an amperite to reduce the voltage to 3.0-3.3. A 30 ohm variable resistance may replace the amperite.

Tube 58 operates from a step-down transformer operated on 110 a.c. and providing a take-off of 2.5 volts. The heater in turn, heats the thoriated cathode which emits an electron flow.

Grid voltage supply. We have used a radio "C" battery or a dry cell. A tap from the "B" or plate battery may be used. The grid has been maintained negative with respect to the cathode.

Plate voltage supply. A radio "B" battery has given good service in all our sets and no attempt has been made to use a rectified a.c. supply. The plate voltage has been kept at a minimum to improve stability of operation.

Screen voltage supply. The positive voltage for the screen grid (tube 58) is obtained from a tap on the plate battery (B-supply). Reduced screen voltage lowers mutual conductance of the tube and results in decreased gain per stage.

CONDITIONS OF OPERATION OF TUBES

Type '32. The '32 type is a screen grid tube, containing a thoriated filament which operates on a low amperage (0.06) consistent with performance. For laboratory purposes, operation is recommended in connection with an air-cell battery. We have satisfactorily employed a single cell of a storage battery. These have been discarded automobile batteries in which at least one cell was operating. No filament resistor is required unless tube is operated from a dry battery or an air-cell battery when a variable resistor or fixed resistor is inserted to give a voltage not to exceed 2.15 volts.

The negative grid bias used was 1.5 volts on the control grid through resistance. The screen is tied back to the plate to improve mutual conductance. To insure a more stable operation, relatively low plate and filament

voltages are used. Maximum stability is obtained when the relationship of the filament, grid and plate potentials is such that the tube is operated so that the tangent to the plate voltage-plate current curve passes through the origin.

The high grid resistance necessitates shielding from electrostatic induction. The interior of the cabinet is lined with heavy lead foil connected with a ground. The tube and grid lead are likewise shielded and grounded.

The '32 type of tube under radio operating conditions is advertised as having a mutual conductance of 650 micromhos and an amplification factor of 780. The base is the medium 4-pin.

Type '99. The '99 type is a triode of low filament amperage (0.063) and operating on a filament voltage of 3.3 volts, maximum. Our voltage was obtained from a discarded automobile battery with two cells operating (4 V). An 'amperite' is inserted in the circuit to reduce the voltage to 3.3 volts. The mutual conductance of this tube is advertised as 425 micromhos and the amplification factor 6.6.

Four and one-half volts have been imposed on the grid through a resistance of seven megohms with a plate potential of 22.5 volts.

Type 58. The 58 is a triple grid super-control amplifier tube with a fifth electrode or suppressor. The tube has an extended mutual conductance operating range. The suppressor has been connected directly with the cathode. The use of 110 volts, alternating current by this tube permits operation from the lighting circuit and eliminates working with storage cells and their attendant charging, adding of distilled water and danger of injury from acid.

The mutual conductance of this tube is advertised as 1600 micromhos and the amplification factor 1280 with a negative grid bias of -3 volts minimum. When the negative grid bias is changed to -50 volts the mutual conductance drops to 2 micromhos. Plate resistance is 800,000 ohms. Heater current 1.0 ampere. The tube has the small 6-pin base with grid on top.

BIOLOGICAL SYSTEM

Oxidation-reduction potentials were observed by using an electrode composed of a short length of spiral platinum-iridium wire sealed into the end of glass tubing which was filled with mercury to make connection with the potentiometer circuit. In all cases, the saturated calomel half-cell was used as a reference electrode.

Potential readings were made on fermentations carried out in 500 cc. or larger Pyrex Erlenmeyer flasks. These flasks were closed with a four-hole rubber stopper which held the platinum electrode, the saturated KCl-agar bridge, gas outlet and siphon sampling tube. The exterior end of the sampling tube was equipped with a pinchcock and was inserted into a plugged sterile test tube.

Before use, the electrode assembly was sterilized by autoclaving in an empty flask. The agar-bridge was carefully filled with hot saturated KCl-agar. Care must be taken to keep the parts sterile and to allow the agar to cool without the formation of bubbles. The electrodes were then placed in freshly inoculated culture flasks.

DESCRIPTION AND USE OF APPARATUS

The materials for the assembly of the vacuum-tube potentiometers described are parts which are readily obtained from dealers in radio equip-

ment or may be made by the experimenter. Special precaution should be used in purchasing variable resistances as it is important to use only those which give positive contact and smooth action. All connections should be soldered with rosin core solder.

APPARATUS EMPLOYING THE '99 TYPE TUBE

The hook-up is given in figure 3. The variable resistances are those of the radio type and need not have the exact resistances as indicated since they are variable. The two resistances R_6 are of the fixed radio type and must be balanced. Values of the magnitude of 10 megohms were found by experiment to be sufficient to reduce polarization of the biological system to a minimum and to permit efficient operation of the apparatus.

Operation. The electron tube potentiometer must first be adjusted so that the galvanometer shows no deflection. With switch S_1 thrown to position 1, the plate resistances R_1 - R_4 are adjusted until the galvanometer reads "zero" when K_1 is closed. Since the plate current may be increased or decreased by a change of potential of the negative grid bias, "C", the unknown source of potential is imposed upon the grid, and the galvanometer indicates any change in the system. In other words, with the vacuum tube "in balance" S_1 is thrown to position 2 so that the potential generated in the potentiometer circuit and opposing biological system being measured, is now balanced by adjustment of the potentiometer until the galvanometer again reads zero. The scale reading gives the voltage of the unknown system. A standard cell is included in the circuit for the purpose of calibrating the potentiometer. To do this the grid-plate circuit is balanced as usual, then S_1 is thrown in position 2, S_2 is "up" and S_3 is thrown to

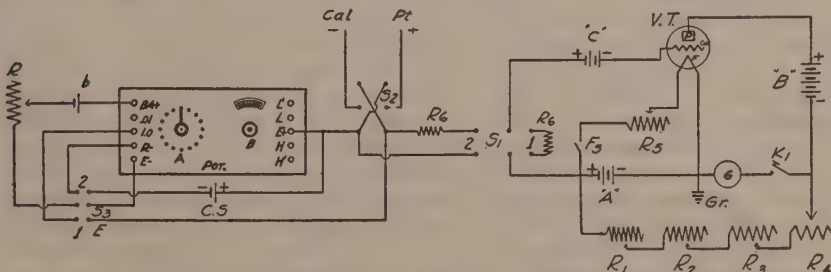


Fig. 3. Electron tube potentiometer using '99 type tube.

Key to Fig. 3

Pot.	Student type Leeds and Northrup Potentiometer
S_1, S_2, S_3	Double-pole, double-throw switches. (S_2 is reversible).
F	Filament switch.
K_1	Tapping key (mercury switch).
"A", "B", "C"	Filament, plate and grid batteries respectively.
b	Potentiometer battery.
R (600 ohms), R_1 (200,000 ohms), R_2 (50,000 ohms), R_3 (50,000 ohms), R_4 (800 ohms), R_5 (30 ohms)	Variable resistances.
R_6	Fixed resistances (10 megohms).
G	Sensitive galvanometer
V.T.	Radio vacuum tube, No. 199 R. C. A.
Cal., Pt. +	Leads to calomel half-cell and platinum (or gold) electrodes.
C.S.	Standard cell.
Gr.	Ground connection.

position 2. The standard cell is protected by a fixed radio resistance of 10,000 ohms. If at the same time K_1 is closed the galvanometer should show no deflection when the potentiometer is set at the voltage of the standard cell. Unless balance is thus obtained the galvanometer auxillary resistance R is adjusted until balance is secured. R is a variable radio resistance mounted on panel. Finally, with the electron tube potentiometer adjusted, O-R and pH readings may be taken. With S_1 in position 2, S_2 in either closed position (by trial, and S_3 in position 1), the potentiometer is again adjusted to zero galvanometer reading and the scale reading gives the required potential.

The parts of the electron tube potentiometer were arranged on a vertical panel of Bakelite and mounted in a discarded radio cabinet on a rectangular wooden base which had been well paraffined. All wiring was heavily insulated and covered with paraffin. Body-capacity effects were largely eliminated by grounding to the water pipe.

APPARATUS EMPLOYING THE '32 TYPE TUBE

This tube is one of the screen grid type and is economical as to current consumption, having a filament rating of 0.06 ampere. Figure 4 gives the set-up of the apparatus using this tube.

The circuit is essentially that of Harrison (1930) in which a galvanometer is used as the null-point instrument. The plate current flowing through the galvanometer is balanced by the compensating current from the filament battery. The biological-potentiometer system is in the grid circuit. To put the set in operation close the "A" battery circuit by means of the filament-resistance switch r_1 and allow the filament to reach a constant temperature (only a few minutes required). At this time switches K and "S" are open. Now close "S," position 1, close s_1 and close mercury switch K . Adjust resistance R until the galvanometer shows zero deflection. When the apparatus is first set up it will be necessary to find some value of resistances r_1, r_2 and r_3 which may need to be cut in by their respective switches s , but when this value is once found for a given tube, R will easily bring about a balance of the plate-filament current through the galvanometer. This resistance will vary somewhat with the individual tube. However, the resistance R , will compensate for the variation due to individual tubes. With the plate circuit balanced, set the potentiometer (Pot) to the reading of the standard cell, set "S" in position 2. Open s_1 and adjust the potentiometer resistance R_h until the galvanometer again reads zero. The usual precautions are to be observed in avoiding polarization of the standard cell. The standard cell has been protected by introducing a radio resistor of 10,000 ohms. The variable resistance R_h is an 800 ohm radio resistor. After the potentiometer is balanced return "S" to position 1. With the biological cell terminals short-circuited there should be no galvanometer deflection when s_1 is opened or closed. The biological system or other source of unknown e.m.f. is connected in the circuit at X so that with s_1 open, the potentiometer is adjusted until the galvanometer reads zero, then from the potentiometer read the unknown potential. We have used a saturated calomel cell as a reference electrode. Platinum, platinum-iridium, or gold electrodes were found satisfactory.

APPARATUS EMPLOYING 58 TYPE TUBE

A.C. radio tubes of the 58 type present a different problem in circuit design than the '32 type. Figure 5 shows the set-up used with this tube.

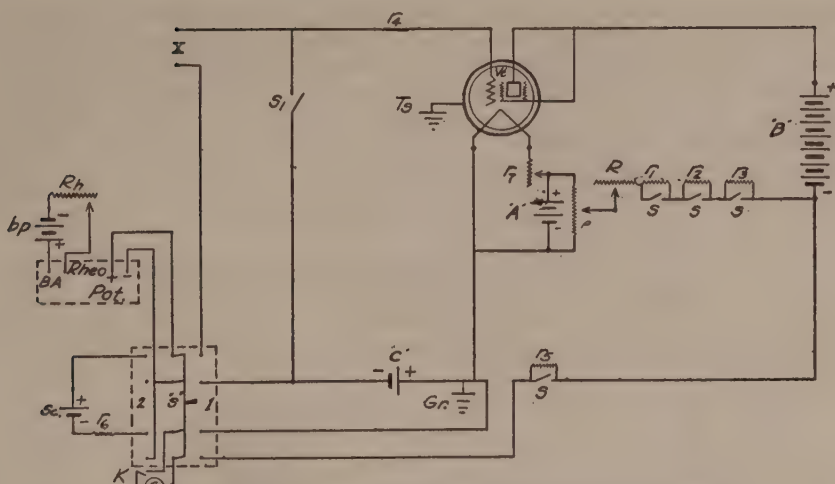


Fig. 4. Electron tube potentiometer, using '32 type of tube.

Key to Figure 4

Vt,	Radio tube '32 type.
"A",	Filament battery, one cell of storage battery or air-cell.
"B",	Plate battery, 45 volt radio battery.
"C",	Grid bias battery, any type of dry battery having 1.5 volt tap.
Pot,	L. and N. student potentiometer.
p,	Radio potentiometer, 400 ohm.
s,	Switches.
s ₁	Grid short-circuiting switch.
K	Mercury switch.
G	Galvanometer.
Gr	Ground connection.
Ts	Tube shield.
r ₁	Filament switch-resistance, 30 ohm.
R	Variable resistances in series. (75,000, 2,500 and 800 ohms)
Bh	Potentiometer resistance, 800 ohm.
bp	Potentiometer battery, 2 dry cells in series.
S	Gang switch (2 double pole-double throw switches tied together).
X	Unknown e.m.f. (biological system).
Sc	Standard cell.
r ₁	Plate resistor. (75,000 ohms)
r ₂	Plate resistor. (50,000 ohms)
r ₃	Plate resistor. (50,000 ohms)
r ₄	Grid resistor, 10 megohms.
r ₅	Protective resistor for galvanometer, 10,000 ohms.
r ₆	Protective resistor for Std. cell, 10,000 ohms.

An ordinary transformer T, as used in A.C. radio sets furnishes the heater current of 2.5 volts. Transformer should not be placed near grid or plate circuits. The "B" and "C" batteries are the same as those used with the '32 tube, but a "D" battery (dry cell) supplies the compensating current through the galvanometer. The additional elements of suppressor and indirectly heated cathode differentiate this tube from the other types. It will be seen from the diagram that the cathode and suppressor are tied together while the screen grid is connected to the "B" battery but at a lower positive value than the plate. The grid circuit contains the potentiometer and biological system in the same manner as in the apparatus using the

'32 type tube. Plug the connector cord into a light socket and allow a few minutes until the cathode reaches a constant temperature. With the grid short-circuiting switch closed and "S" set to position 1 adjust R until the galvanometer reads zero when K is closed. Set "S" at position 2 and adjust the potentiometer to the standard cell in the usual way. The apparatus is now ready to test for the unknown e.m.f. This is done in the same manner as with the '32 type apparatus.

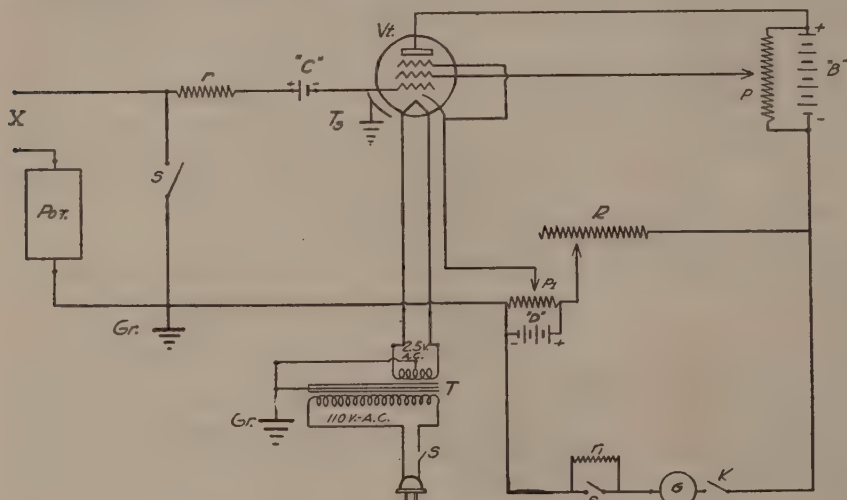


Fig. 5. Electron tube potentiometer using 58 A. C. tube

Key to Figure 5

Vt	58 Radio tube.
T	Step-down transformer for 110 volts, primary with 2.5 volt secondary tap. Shielded and grounded.
"B"	Radio plate battery 45 volts.
"C"	Grid battery 1.5 to 4.5 volts.
"D"	Compensating plate current battery, dry cell.
Pot	L. and N. student potentiometer.
G	Galvanometer.
s	Switches.
K	Mercury switch.
R	Variable resistances in series, 75,000, 25,000, 5,000 and 800 ohms.
p	Potentiometer, radio type, 5,000 ohms.
p ₁	Potentiometer, radio type, 3,000 ohms.
Gr	Ground.
Ts	Tube shield.
r	Grid resistor. (3,000 ohms)
r ₁	Galvanometer protective resistance, 10,000 ohms.
X	Unknown source of e.m.f. (culture).

USE OF MERCURY SWITCH

It has been our experience that the ordinary tapping key in the galvanometer circuit is the cause of fluctuations of the galvanometer needle. These variations are caused by the varying pressure in changing the contact resistance of the key. To overcome this difficulty a mercury switch was constructed as shown in the diagram figure 6. The outer vessel was a large test tube having a platinum wire Pt, sealed in the bottom. About two centimeters from the bottom of this tube a short side-arm was sealed



Fig. 6. Mercury switch

on. This side-arm also had a platinum wire terminal. A second but smaller test tube was used as a plunger or piston. Mercury was placed in the bottom of the larger tube until it supported the piston but failed to fill the side arm. It is now clear that by depressing the piston *f*, the mercury rises and makes contact through the platinum leads. This switch serves as a tapping key and also as a closed circuit switch since the shoulder of the piston at *a*, may be engaged with the lip of the outer vessel at *b*. As the galvanometer is equipped with a protective resistance and since the current through the galvanometer is too small to injure the instrument when approximate balance is found, *K* may remain closed.

USE OF SINGLE GANG MULTIPLE SWITCH

Figure 7 shows a single gang multiple switch which has been of service in making series of determinations. The redox potentials of 15 fermentations can be made rapidly and without disturbing the flasks by setting the switch to the numbers corresponding to the flasks and making the readings in the usual manner. The saturated calomel cell is connected to the saturated KCl-agar electrode by means of a small rubber tube filled with saturated KCl solution. Glass t-tubes are inserted at intervals in the rubber tubing to provide leads to the individual KCl-agar electrodes. A switch is provided to throw out the biological systems. The common electrical connection to the platinum electrode is shown in figure 7. A similar multiple switch built into the apparatus is shown in plate I near the upper left hand corner of the instrument. The switch shown

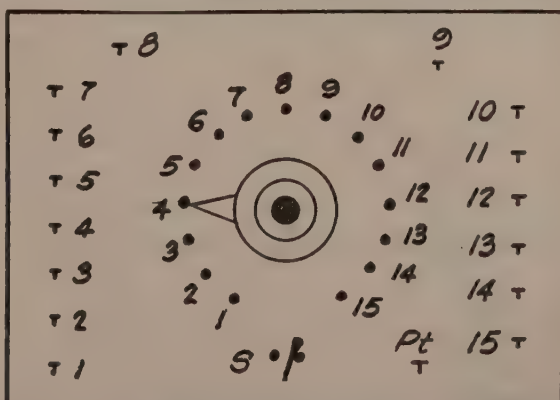


Fig. 7. Multiple switch. Pt. is the common connection to the platinum electrode terminal on set. S is a switch.

in figure 7 was constructed of Bakelite using brass screws with the heads ground down for terminals.

SUMMARY

Three electron tube potentiometers suitable for the determination of redox potentials in bacterial cultures have been described. The apparatus uses inexpensive electron tubes obtainable from radio dealers. The apparatus has been in use for several months and gives consistent and reproducible readings.

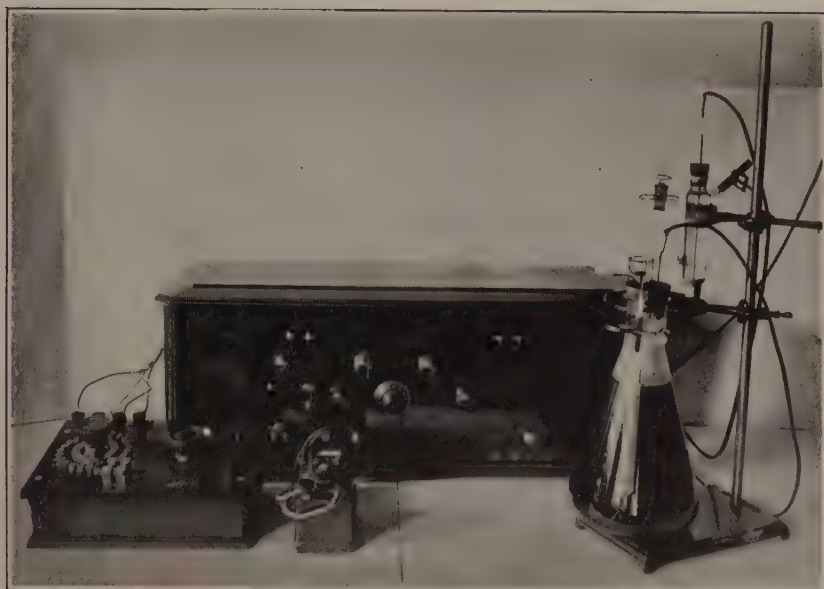
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Plate I

Electron tube potentiometer using '58 type A. C. tube

Plate I



THE IOWA FLORA

AN ANNOTATED LIST OF THE FERNS, FERN ALLIES AND THE NATIVE
AND INTRODUCED FLOWERING PLANTS OF THE STATE REPRESENTED
IN THE IOWA STATE COLLEGE HERBARIUM

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From the Department of Botany, Iowa State College

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INTRODUCTION

The following contribution from the State College Herbarium at Ames, Iowa, has been prepared for a three-fold purpose. First, to place on record a list of the Iowa species at present in the Herbarium; second, to serve as a guide to systematic students of our flora; and third, to enable collectors and correspondents to supply native and introduced plants not already represented in the collection by Iowa specimens.

The value of a good workable Herbarium for ready reference to any institution of learning where botany is included in the courses, cannot be questioned, and this is more especially true of one which is more or less closely connected with forestry and agriculture.

This Herbarium was begun about 1870 by Dr. C. E. Bessey, then Head of the Department of Botany. Its growth was comparatively slow until after Dr. L. H. Pammel became Head of the Department in February, 1889, a position which he held for forty years. At the time Dr. Pammel came to the institution the collection numbered about 10,000 specimens. Since then its growth has been very rapid. The gift of his and the writer's private herbaria, the purchase of the Parry and a part of the Holway herbaria, the gift of a large and valuable grass collection by Dr. A. S. Hitchcock, the gift or purchase of numerous smaller collections, to which has been added the results of extensive field work by Dr. Pammel and other members of the staff, with numerous exchanges, have all combined to form a very valuable collection. Besides the plants listed in this paper, the Herbarium also contains an extensive and valuable collection of algae, the parasitic and other fungi, the lichens, hepatics, mosses and other forms of plant life, all of which fill a place in Nature's plan, and the study and understanding of which does much to broaden one's concept of the myriad forms of vegetable life.

The flora of Iowa, while not as rich in species as that of some states having a more diversified topography, still compares quite favorably with the surrounding Plain States. Ours is a rapidly changing flora, since an unusually large proportion of our area consists of cultivated land, thus destroying many of our rarer prairie plants, while the draining of our marshes and shallow lakes, has greatly reduced the aquatic flora, which in pioneer days was so well represented. Then, too, the immigrant flora, largely European in origin, is increasing every year, numbering at present about 294 species, many of them troublesome weeds.

While Iowa is largely a prairie state, yet its trees and shrubs are of considerable importance. The trees are represented by about 77 native and 15 introduced species which have escaped from cultivation; the shrubs and woody vines by 109 native and 8 escapes.

Three genera of the rose family, *Crataegus*, *Rubus* and *Rosa*—the thorn apples, brambles, and wild roses, are of great taxonomic difficulty, and have never been worked up satisfactorily. This is partly owing to the fact that the specimens available for study, are not sufficiently complete, with flowers, leaves and mature fruit, to enable one in many cases to make a positive identification. Even with good material on hand, there are so many intermediate forms that these groups are very puzzling to every one who undertakes to understand them.

The family names and their sequence in this paper follow Engler and Prantl, the system used in the Herbarium. Since there is no uniformity in the sequence of the genera and species as used by our modern systematists, that of Gray's Manual, 7th edition, has been quite closely followed, as it is more extensively used in our state than any other text. However, recent changes in nomenclature and the International Rules have made numerous changes in plant names seem advisable, but, where these occur, they are indicated by the synonyms which have been freely admitted. In this matter of accepted names the author has endeavored to be conservative.

The rarity or abundance of each species has been indicated in a general way, as well as its geographical distribution. When any plant listed is very rare, or is represented in the Herbarium by a single Iowa specimen, it is usually followed by the collector's name in parenthesis.

The numbers preceding the names of the families of the flowering plants are those used in the Herbarium index, and are given for convenience of reference for those using the Herbarium. For the same reason, also, each species is given a serial number, and each introduced plant is preceded by an asterisk, and followed by the native habitat in parenthesis.

The author acknowledges his indebtedness for many favors to the late Dr. L. H. Pammel, to Dr. I. E. Melhus—the present Head of the Department of Botany, and to other members of the staff who, in various ways, have been of great assistance. Thanks are also due numerous specialists who at various times have named or verified critical specimens. Among these latter are the late Dr. P. A. Rydberg of the New York Botanic Garden, Dr. A. S. Hitchcock and Mrs. Agnes Chase of the U. S. National Herbarium, Dr. C. R. Ball, the eminent authority on willows, Dr. B. L. Robinson, and other specialists at the Gray Herbarium, Mr. B. F. Bush of Courtney, Mo., Miss M. Rae Johns of Davenport and others. The author is also indebted to Miss Charlotte M. King for proof-reading and to Dr. J. C. Gilman for his critical examination of the manuscript, all of which is gratefully acknowledged.

That the Iowa State College Herbarium may contain a more nearly complete representation of our flora, it is earnestly requested that anyone finding either native or introduced plants growing within our boundary and not listed in this paper, will send good specimens, suitable for mounting, with the locality, date and name of the collector.

In conclusion it is hoped that this contribution may be of some assistance to students of our flora, and plant lovers in general, and help to awaken an interest in the living things around us.

R. I. CRATTY
Curator

Department of Botany
Iowa State College
Sept. 1, 1932

PTERIDOPHYTA

FERNS AND FERN ALLIES

OPHIOGLOSSACEAE (*Adder's Tongue Family*)**Botrychium** Sw. Moonwort

1. *B. obliquum* Muhl. Rare; Van Buren Co. (E. W. Graves)
2. *B. ternatum intermedium*, D. C. Eaton. (*B. silaifolium* Presl) Very rare; only one plant found; Fayette Co., (Bruce Fink)
3. *B. dissectum* Spreng. Rare; Van Buren Co., (E. W. Graves)
4. *B. virginianum* (L.) Sw. Grape Fern, Rattlesnake Fern. Frequent in rich woods.

OSMUNDACEAE (*Flowering Fern Family*)**Osmunda** (Tourn.) L.

5. *O. regalis* L. Royal Fern. Infrequent.
6. *O. cinnamomea* L. Cinnamon Fern. Infrequent.
7. *O. Claytoniana* L. Clayton's Fern. Our commonest species of the genus.

POLYPODIACEAE (*Fern Family*)**Onoclea** L.

8. *O. sensibilis* L. Sensitive Fern. Frequent throughout in moist woods.

Pteretis Raf.

9. *P. nodulosa* (Michx.) Nieuwl. (*Onoclea Struthiopteris* of older manuals, but now considered distinct from the European plant, *P. Struthiopteris* Nieuwl.) Ostrich Fern. Infrequent in rich woods.

Woodsia R. Br.

10. *W. scopulina* D.C. Eaton. Very rare; Gitchie Manitou State Park, Lyon Co. (Shimek, Cratty and Melhus).
11. *W. obtusa* (Spreng.) Torr. Frequent in rocky ravines; central and eastern sections.

Filix Adans.

12. *F. bulbifera* (L.) Underw. (*Cystopteris* Bernh.) Frequent in the eastern part.
13. *F. fragilis* (L.) Underw. (*Cystopteris* Bernh.) Very common in rich, open woods.

Polystichum Roth.

14. *P. acrostichoides* (Michx.) Schott. (*Aspidium* Sw.) Christmas Fern. Frequent in the eastern part.

Phegopteris (Presl.) Fée. Oak Fern, Beech Fern.

15. *P. polypodioides* Fée. (*Dryopteris* C. Chr.) Frequent in the northeastern part.
16. *P. hexagonoptera* (Michx.) Fée. (*Dryopteris* C. Chr.) Frequent in the eastern half.
17. *P. Dryopteris* (L.) Fée. (*Dryopteris* Britton) Infrequent northeast.

18. *P. Robertiana* (Hoff.) A. Br. (*P. calcarea* Fee, *Dryopteris* C. Chr.) Rare on limestone rocks; northeastern.

Dryopteris Adans.

19. *D. Thelypteris* (L.) A. Gray. (*Aspidium* Sw.) Frequent in low ground in the eastern part.
 20. *D. Goldiana* (Hook.) A. Gray. (*Aspidium* Hook.) Shield Fern. Infrequent.
 21. *D. cristata* (L.) A. Gray. Rare; Muscatine Co. (Ferd Reppert).
 22. *D. marginalis* (L.) A. Gray. (*Aspidium* Sw.) Evergreen Wood Fern. Infrequent; Hardin and Allamakee counties.
 23. *D. spinulosa* (Muell.) Kuntze. (*Aspidium* Sw.) Frequent in the eastern part and quite variable.

Camptosorus Link.

24. *C. rhizophyllus* (L.) Link. Walking Fern. Frequent in rocky, shady ravines.

Asplenium L.

25. *A. platyneuron* (L.) Oakes. Ebony spleenwort. Very rare; a small colony near Copras Creek, Van Buren Co. (E. W. Graves) and Lee Co. (Jess L. Fults).
 26. *A. pycnocarpon* Spreng. (*Asplenium angustifolium* Michx.) Rare in the northeastern part.

Athyrium Roth.

27. *A. thelypteroides* (Michx.) Desv. (*Asplenium acrostichoides* Sw.) Infrequent.
 28. *A. angustum* (Willd.) Presl. (*Asplenium Filix-foemina* of our older Manuals not of L.) Lady Fern. Very common in rich, shady woods.

Adiantum L.

29. *A. pedatum* L. Maidenhair Fern. Very common in rich woods.

Pteridium Scop.

30. *P. aquilinum* (L.) Kuhn. (*Pteris* L.) Brake or Bracken. Widely distributed but common only locally.

Cryptogramma R. Br.

31. *C. Stelleri* (S.G. Gmelin) Prantl. (*Pellaea gracilis* Hook.) Infrequent; Webster Co. and northeastward.

Pellaea Link.

32. *P. glabella* Mett. (*P. atropurpurea* in part of authors). Northern cliff Brake. Frequent on calcareous rocks, especially in the eastern part.
 33. *P. atropurpurea* (L.) Link. Southern Cliff Brake. Very rare in the southeastern part. Van Buren Co. (E. W. Graves).

Cheilanthes Sw.

34. *C. Féei* Moore. (*C. lanuginosa* Nutt.) Infrequent; northeastern.

Polypodium (Tourn.) L.

35. *P. virginianum* L. (Not *P. vulgare* L.) Polypod. Common in rocky woods; a variable species.

SALVINIACEAE

Azolla Lam.

36. *A. caroliniana* Willd. Rare; Fremont and Allamakee counties.

EQUISETACEAE (*Horse-tail Family*)**Equisetum** (Tourn.) L.

37. *E. arvense* L. Small Scouring Rush. Very common in low ground.
38. *E. pratense* Ehrh. Resembling the preceding. Rare in the eastern part.
39. *E. sylvaticum* L. Rare; Jasper and Chickasaw counties.
40. *E. fluviatile* L. (*E. limosum* L.) Frequent in our northern marshes.
41. *E. robustum* A. Br. (*E. hyemale* var. A. A. Eaton) Large Scouring Rush. Common, especially on dry banks of streams.
42. *E. kansanum* Schaffner. Frequent throughout in rather dry situations. Formerly confused with the next.
43. *E. laevigatum* A. Br. Frequent on rather dry prairies; the cones pointed.

LYCOPODIACEAE (*Club Moss Family*)**Lycopodium** L.

44. *L. lucidulum* Michx. Dubuque, Hardin, Chickasaw and Allamakee counties. Infrequent in cool, damp woods.
45. *L. obscurum* L. (*L. obscurum dendroideum* A. A. Eaton) Very rare; Jones Co. (E. E. Reed).
46. *L. complanatum* L. Ground Pine. Frequent in the eastern counties.

SELAGINELLACEAE

Selaginella Beauv.

47. *S. rupestris* (L.) Spreng. Rare; Allamakee, Chickasaw and Lyon counties.

SPERMATOPHYTA

SEED-BEARING PLANTS

GYMNOSPERMAE

3. TAXACEAE (*Yew Family*)**Taxus** (Tourn.) L. Yew

48. *T. canadensis* Marsh. Frequent in the northeastern section.

4. PINACEAE (*Pine Family*)**Pinus** (Tourn.) L.

49. *P. Strobus* L. (*Strobus Strobus* Small) White Pine. Rare; Hardin and eastern counties.

Abies (Tourn.) Hill.

50. *A. balsamea* (L.) Mill. Balsam Fir. Infrequent. Winneshiek and Allamakee counties.

Juniperus (Tourn.) L.

51. *J. communis* L. Common Juniper. Infrequent in the northeastern section.
52. *J. virginiana* L. Red Cedar. Common and also frequent in cultivation and as an escape.
53. *J. horizontalis* Moench. Creeping Juniper. Very rare; Floyd county.

ANGIOSPERMAE

6. TYPHACEAE (*Cat-tail Family*)**Typha** (Tourn.) L.

54. *T. latifolia* L. Cat-tail Flag. A common plant in shallow water.

8. SPARGANIACEAE (*Bur-reed Family*)**Sparganium** (Tourn.) L. Bur-reed.

55. *S. eurycarpum* Engelm. Large Bur-reed. Our commonest large-fruited species in shallow water.
 56. *S. androcladum* (Engelm.) Morong. (*S. americanum* var. Fern. & Eames). Rare in shallow water.
 57. *S. americanum* Nutt. (*S. simplex Nuttallii* Engelm.) Infrequent.
 58. *S. chlorocarpum* Rydb. (*S. simplex* in part, of Gray's Manual, 6th Ed.) Rare; Emmet and Story counties.
 59. *S. angustifolium* Michx. Rare; Story Co. (Pearl Clayton)

9. POTAMOGETONACEAE (*Pond-weed Family*)**Potamogeton** (Tourn.) L. Pondweed.

60. *P. natans* L. Frequent in shallow lakes and ponds.
 61. *P. amplifolius* Tuck. Frequent in shallow water. A large leaved species.
 62. *P. epihydrus* Raf. (*P. Nuttallii* Cham. & Schlecht.) Rare in ponds.
 63. *P. americanus* Cham. & Schlecht. (*P. lonchites* Tuck). Frequent in streams.
 64. *P. heterophyllus* Schreb. Frequent in northern marshes.
 65. *P. illinoensis* Morong. Infrequent; ponds and streams, northern and eastern sections.
 66. *P. praelongus* Wulf. Rare; usually in quite deep water in lakes.
 67. *P. Richardsonii* (Benn.) Rydb. (*P. perfoliatus* var. Benn.) Frequent in lakes and ponds; our most beautiful species.
 68. *P. compressus* L. (*P. zosteræfolius* Schum.) Frequent in lakes and ponds.
 69. *P. foliosus* Raf. Frequent; the var. *niagarensis* Tuck. is a rank form occurring in running water.
 70. *P. Friesii* Ruprecht. Infrequent in shallow lakes.
 71. *P. pusillus* L. Frequent; a delicate species in still water.
 72. *P. dimorphus* Raf. (*P. spirillus* Tuck.) Rare in ponds.
 73. *P. pectinatus* L. Common.
 74. *P. interruptus* Kitaibel. Rare; Lake Okoboji, Dickinson Co. (F. W. Paige).

Zannichellia (Mich.) L.

75. *Z. palustris* L. Horned Pondweed. Rare in shallow water, margins of lakes. The var. *pedunculata* J. Gay, occurs in Muscatine Co. (Barnes and Miller).

10. NAJADACEAE

Najas L. Naiad.

76. *N. flexilis* (Willd.) Rost. & Schmidt. Frequent in shallow water of lakes and ponds.

12. JUNCAGINACEAE

Triglochin L. Arrow-grass.

77. *T. palustris* L. Very rare in a bog in Emmet Tp. Emmet Co. (B. O. Wolden).
 78. *T. maritima* L. Rare in our northern marshes.

Scheuchzeria L.

79. *S. palustris* L. Very rare in peat bogs, Emmet Co. (R.I. Cratty, F.W. Paige).

13. ALISMACEAE (*Water Plantain Family*)**Alisma** L. Water Plantain.

80. *A. subcordatum* Raf. (*A. plantago-aquatica* of Am. authors, not of L.) Very common in low wet ground.

Echinodorus Richard.

81. *E. cordifolius* (L.) Griseb. Infrequent in shallow water.
 82. *E. radicans* (Nutt.) Engelm. Rare in similar situations.

Lophotocarpus Th.Durand.

83. *L. calycinus* (Engelm.) J.G. Smith. Rare in Muscatine and Emmet counties; the lower flowers are perfect—the plant is apt to be confused with *Sagittaria*.

Sagittaria L. Arrow-head.

84. *S. cuneata* Sheldon (*S. arifolia* Nutt.) Common in marshes.
 85. *S. latifolia* Willd. Very common in marshes, and shallow water. The tubers of this plant were the *Wab-es-i-pin-ig*, or Swan Potato of the Chippewa Indians.
 86. *S. rigida* Pursh. (*S. heterophylla* Pursh not Schreb.) Infrequent.
 87. *S. cristata* Engelm. Very rare in shallow water. The type collected in a small lake east of Armstrong, Emmet Co., since drained. (R.I. Cratty). Also in Minnesota.
 88. *S. graminea* Michx. Rare in the southern half of the state.

15. HYDROCHARITACEAE

Philotria Raf.

89. *P. ioensis* Wylie. (*Elodea canadensis* in part of American authors). Frequent; ponds and slow streams.

Vallisneria (Mich.) L.

90. *V. spiralis* L. Tape-grass. Eel-grass. Wild or Water Celery; a favorite food of the canvas-back duck. Frequent in quiet water.

17. GRAMINACEAE (*Grass Family*)**Tripsacum** L.

91. *T. dactyloides* L. Gama-grass. Frequent in low ground in the southern counties.

Andropogon (Roen) L.

92. *A. scoparius* Michx. (*Schizachyrium* Nash). Common on up-land prairies.

93. *A. furcatus* Muhl. Blue-stem, Blue-joint. Very common; border of woods and on prairies.

*94. *A. Hallii* Hack. Rare; escaped from cultivation in Muscatine Co. (Western U.S.).

Sorghastrum Nash.

95. *S. nutans* (L.) Nash. Indian-grass. A common and beautiful species.

Sorghum L.

*96. *S. halepense* (L.) Pers. Johnson-grass. Escaped in a few localities. *S. vulgare* Pers. Sorghum, has been collected as an escape in Muscatine Co. (Europe).

Digitaria Scop. (*Syntherisma* Walt.)

97. *D. filiformis* (L.) Koeler. Rare; Muscatine Co. (A. A. Miller).

*98. *D. Ischaemum* (Schreb.) Nash. (*Panicum lineare* Krock.) Smaller crab-grass; a common weed, especially in lawns. (Europe).

*99. *D. sanguinalis* (L.) Scop. (*Panicum* L.; *Syntherisma* Dulac.) Large crab-grass. Common; a bad weed in gardens. (Europe).

Leptoloma Chase.

100. *L. cognatum* (Schultes) Chase. (*Panicum autumnale* Bosc.) Infrequent in dry soil.

Paspalum L.

101. *P. stramineum* Nash. Frequent in sandy soil; eastern and southern.

102. *P. pubescens* Muhl. Very rare; a new addition to our grass flora. Lee Co. (Jess L. Fufts).

Echinochloa Beauv.

*103. *E. Crus-galli* (L.) Beauv. Barnyard grass. Common in cultivated soil, and waste places. (Europe).

*104. *E. Walteri* (Pursh) Nash. (*Panicum Crus-galli hispidum* Torr.) Infrequent in wet places. (Eastern and southern U.S.).

Panicum L. Panic-grass.

105. *P. capillare* L. Old witch-grass. Very common.

*106. *P. miliaceum* L. Broom-corn Millet. An occasional escape. (Europe).

107. *P. dichotomiflorum* Michx. A common weedy species, rooting at the nodes.

108. *P. virgatum* L. A very common and valuable grass.

109. *P. depauperatum* Muhl. Frequent in rather dry soil.

110. *P. perlongum* Nash. Infrequent; Emmet Co. (Cratty, Wolden) Too near the preceding.

111. *P. huachucae* Ashe. Common on dry prairies.

112. *P. huachucae silvicola* Hitch. & Chase. Frequent, woods and clearings.

113. *P. implicatum* Scribn. Rare.

114. *P. tennesseense* Ashe. Rare.

115. *P. praecocius* Hitch. & Chase. Frequent on our northern morainic hills.

116. *P. Scribnerianum* Nash. Frequent and variable. This species closely resembles the next.

117. *P. Leibergeri* (Vasey) Scribner. Frequent.

118. *P. Wilcoxianum* Vasey. Rare.

119. *P. latifolium* L. Frequent.

Setaria Beauv. (*Chaetochloa* Scribner).

*120. *S. glauca* (L.) Beauv. Yellow foxtail. A common, weedy species. (Europe).

*121. *S. verticillata* (L.) Beauv. Bristly foxtail. A disagreeable, weedy species, becoming common. (Europe).

*122. *S. viridis* (L.) Beauv. Green Foxtail. Very common in cultivated fields. (Europe).

*123. *S. italica* (L.) Beauv. Millet or Hungarian Grass. Cultivated and rarely spontaneous. (Europe).

*124. *S. italica germanica* (Mill.) Richter. German millet. Less common in cultivation, and occasionally spontaneous. (Europe).

Cenchrus L. Sand-bur.

125. *C. pauciflorus* Benth. (*C. tribuloides* of authors in part, not of L.) A very disagreeable, weedy species; frequent in dry ground.

Zizania L. Wild or Indian Rice.

126. *Z. aquatica* L. (*Z. interior* Rydb.) Frequent in our northern marshes.

127. *Z. palustris* L. A narrow-leaved form of the preceding, and less frequent.

Leersia Sw. (*Homalocenchrus* Mieg.)

128. *L. virginica* Willd. Common in low, wet woods.

129. *L. oryzoides* (L.) Swz. Rice Cut-grass. Common in shallow water, or very wet soil.

130. *L. lenticularis* Michx. Infrequent. Mostly in the eastern part.

Phalaris L.

*131. *P. canariensis* L. Canary Grass. An occasional escape. (Europe).

132. *P. arundinacea* L. Wild Canary Grass. Frequent in wet ground.

Hierochloë (Gmelin) R. Br.

133. *H. odorata* (L.) Wahl. (*H. borealis* R. & S.; *Savastana odorata* Scribner) Holy or Vanilla Grass. Frequent in low, wet soil.

Oryzopsis Michx.

134. *O. racemosa* (Smith) Ricker. Mountain Rice. Frequent in rich woods.

Stipa L.

135. *S. viridula* Trin. Frequent in the western part.

136. *S. comata* Trin. & Ruprecht. Infrequent in dry soil in western part.

137. *S. spartea* Trin. Porcupine Grass. Common in dry prairie soil.

Aristida L.

138. *A. basiramea* Engelm. Infrequent.

139. *A. gracilis* Ell. Rare; Lee Co. (A. S. Hitchcock).

140. *A. intermedia* Scribn. & Ball. Infrequent; Audubon and Pottawattamie counties.

141. *A. oligantha* Michx. Poverty Grass. Our commonest species, especially in the southeastern part, where it is very troublesome in sheep pastures.

142. *A. tuberculosa* Nutt. Rare; Muscatine Co.
 143. *A. longiseta robusta* Merrill. Very rare; Plymouth Co. (John Leibig).

Muhlenbergia Schreb. Drop-seed Grass.

144. *M. sobolifera* (Muhl.) Trin. Infrequent.
 145. *M. Torreyi* Hitch. (*M. sylvatica* Torr., *M. umbrosa* Scribn.) Infrequent in moist woods.
 146. *M. mexicana* (L.) Trin. A common, persistent grass in wet ground.
 147. *M. racemosa* (Michx.) BSP. (*M. glomerata* Trin.) Common.
 148. *M. Schreberi* J. F. Gmelin. A diffuse species, frequent in wet ground.
 149. *M. cuspidata* (Torr.) Rydb. (*Sporobolus* Torr.) Frequent on dry knolls.

Brachyelytrum Beauv.

150. *B. erectum* (Schreb.) Beauv. Frequent in rich woods.

Phleum L.

- *151. *P. pratense* L. Timothy. Very common in cultivation and as an escape. (Europe).

Alopecurus L. Wild Foxtail.

- *152. *A. pratensis* L. Rare as an escape. (Europe).
 153. *A. geniculatus* L. Common in wet ground.
 154. *A. aristulatus* Michx. (*A. geniculatus* var. Torr.) Frequent in similar situations.

Sporobolus R. Br. Drop-seed or Rush Grass.

155. *S. asper* (Michx.) Kunth. Frequent on hillsides.
 156. *S. vaginiflorus* (Torr.) Wood. Frequent; resembling the next but larger.
 157. *S. neglectus* Nash. A worthless, annual species, common in pastures.
 158. *S. cryptandrus* (Torr.) Gray. Infrequent.
 159. *S. heterolepsis* Gray. A common species on upland prairie soil.
 *160. *S. asperifolius* (Nees. & Meyer) Thurber. Rare; R.R. right-of-way, two miles east of Nevada, Story Co. Undoubtedly introduced. (Western Plains).

Cinna L. Wood Grass.

161. *C. arundinacea* L. Common in low woods.

Agrostis L.

- *162. *A. alba* L. (*A. vulgaris* Host.) Red-top. Common in cultivation and as an escape. (Europe).
 163. *A. hyemalis* (Walt.) BSP. Hair Grass. Common.
 164. *A. perennans* (Walt.) Tuck. Infrequent, a variable species.

Calamagrostis Adans. Reed Bent-grass.

165. *C. canadensis* (Michx.) Beauv. Very common in low, wet ground.
 166. *C. Macouniana* Vasey. Rare in marshes, Emmet Co. (R. I. Cratty).
 167. *C. inexpansa* Gray. (*C. confinis* Kunth.) Frequent in our northern marshes.

Calamovilfa Hack.

168. *C. longifolia* (Hook.) Hack. (*Calamagrostis* Hook.) Frequent in dry, sandy ground.

Holcus L.

- *169. *H. lanatus* L. Velvet Grass. An occasional escape. (Europe).

Avena (Tourn.) L. Oat.

- *170. *A. fatua* L. Frequent as a weed in grain fields and waste places. (Europe).
 *171. *A. sativa* L. The cultivated oat; occasionally adventive. (Eurasia).

Arrhenatherum Beauv. Oat Grass.

- *172. *A. elatius* (L.) Beauv. Rare; adventive in waste places. (Europe).

Danthonia DC. Wild Oat Grass.

173. *D. spicata* (L.) Beauv. Infrequent in dry, sterile soil.

Cynodon L.

- *174. *C. Dactylon* (L.) Pers. (*Capriola* Kuntze). Bermuda Grass. An occasional introduction in lawns; not hardy in this latitude. (Europe).

Spartina Schreb. Cord or Slough Grass.

175. *S. Michauxiana* Hitch. (*S. cynosuroides* of Gray's Manual in part, not of Roth *S. pectinata* Bosc?) Common on low prairies.

Schedonnardus Steud.

176. *S. paniculatus* (Nutt.) Trel. (*S. texanus* Steud.) Very rare on a barren hilltop, Guthrie Co. (L. R. Miller).

Bouteloua Lag. Mesquite Grass.

177. *B. gracilis* (HBK.) Lag. (*B. oligostachya* (Nutt.) Torr). Rare in dry ground, northwest section.
 178. *B. hirsuta* Lag. Frequent in dry ground; resembling the preceding.
 179. *B. curtipendula* (Michx.) Torr. (*B. racemosa* Lag.) A common valuable upland species.

Beckmannia Host.

180. *B. erucaeformis* (L.) Host. (*B. Syzigachne* Fern.) Infrequent in low ground.

Eleusine Gaertn.

- *181. *E. indica* (L.) Gaertn. Yard Grass. Infrequent. Introduced from the Old World Tropics. (S. Asia).

Diplachne Beauv.

182. *D. fascicularis* (Lam.) Beauv. (*Leptochloa* Gray, *D. acuminata* Nash). Rare; Webster Co. (F. W. Paige, J. C. Blumer).

Buchloe Engelm.

183. *B. dactyloides* (Nutt.) Engelm. (*Bulbilis* Raf.) Buffalo Grass. Very rare in dry soil; Lyon Co. (B. Shimek).

Phragmites Trin. Reed Grass.

184. *P. communis* Trin. Frequent in marshy places; the plumes in fruit very conspicuous.

Triodia R. Br.

185. *T. flava* (L.) Hitch. (*Tridens* Hitch.; *Tricuspis seslerioides* Torr.) Infrequent in the southern part.

Triplasis Beauv.

186. *T. purpurea* (Walt.) Chapm. Frequent southeast in sandy soil.

Eragrostis Beauv.

187. *E. hypnoides* (Lam.) BSP. Common in sandy soil; lake shores and banks of streams.

- 188. *E. Frankii* Steud. Frequent; sandy banks of lakes and streams.
- 189. *E. pilosa* (L.) Beauv. (*E. Purshii* Schrad.) Very common in dry, sandy soil.
- *190. *E. cilianensis* (All.) Link. (*E. megastachya* Link.; *E. major* Host.) A very common, ill smelling weedy species in cultivated soil. (Europe).
- *191. *E. minor* Host. Infrequent; resembling the preceding, but the spikes much smaller. (Europe).
- 192. *E. trichodes* (Nutt.) Nash. (*E. tenuis* Gray). Rare; Muscatine Co.
- 193. *E. pectinacea* (Michx.) Steud. Frequent in dry, sandy soil; our handsomest species.
- *194. *E. suaveolens* Becker. (*E. collina* Trin.) Rare as an escape from cultivation; Story Co. (Russia).

Sphenopholis Scribn.

- 195. *S. obtusata* (Michx.) Scribn. Frequent; dry prairies and morainic hills.
- 196. *S. pallens* (Spreng.) Scribn. (*Eatonia pennsylvanica* Gray) Frequent in rather low ground.

Koeleria Pers.

- 197. *K. cristata* (L.) Pers. Common on upland prairies.

Melica L. Melic Grass.

- 198. *M. nutica* Walt. Common in woods.
- 199. *M. nitens* Nutt. (*M. diffusa* of authors, not of Pursh) Frequent.
- 200. *M. Porteri* Scribn. Rare; Johnson Co. (Fitzpatrick).

Diarrhena Beauv.

- 201. *D. diandra* (Michx.) Wood. Frequent in the southern part.

Distichlis Raf.

- 202. *D. spicata* (L.) Greene. (*D. stricta* Rydb.) Rare; a saline coast species, but rare in the interior; Ida Co. (J. S. Crawford).

Dactylis L.

- *203. *D. glomerata* L. Orchard Grass. Common; introduced from Europe.

Poa L. Spear Grass, Meadow Grass.

- *204. *P. annua* L. Common as an escape. (Europe).
- 205. *P. Chapmaniana* Scribn. Infrequent.
- *206. *P. compressa* L. Canada Blue Grass. Very common. (Europe).
- 207. *P. palustris* L. (*P. triflora* Gilib., *P. serotina* Ehrh.) Common in low ground.
- *208. *P. pratensis* L. Kentucky Blue Grass. Very common in cultivation and as an escape. (Europe).
- 209. *P. sylvestris* Gray. Infrequent; Emmet and Hardin counties.
- 210. *P. debilis* Torr. Infrequent in the southeastern part.
- 211. *P. Wolfii* Scribn. Rare on limestone rocks; Allamakee Co.

Fluminia Fries

- 212. *F. arundinacea* (Willd.) Hitch. (*Scolochloa festuacea* Link.) Rare in our northern marshes; Emmet and Webster counties. (R. I. Cratty).

Glyceria R. Br. Manna Grass.

- 213. *G. nervata* (Willd.) Trin. Fowl Meadow Grass. Common in low ground or shallow water.
- 214. *G. grandis* S. Wats. A tall, robust species common in shallow water.
- 215. *G. borealis* (Nash) Batchelder. Common in low marshy ground.

Festuca L. Fescue Grass.

- 216. *F. octoflora* Walt. (*F. tenella* Willd.) Frequent in dry, sterile soil.
- *217. *F. rubra* L. Introduced with lawn grass; an undesirable species. (Europe).
- *218. *F. ovina* L. Sheep Fescue. Sparingly introduced. Emmet Co. (B. O. Wolden) (Europe).
- *219. *F. elatior* L. A common escape. (Europe).
- 220. *F. obtusa* Spreng. (*F. nutans* Spreng., not Moench.) Common in low rich woods.
- 221. *F. Shortii* Kunth. Frequent; low woods and prairies.

Bromus L. Brome Grass.

- *222. *B. secalinus* L. Chess or Cheat. A frequent weed in grain fields. (Eurasia).
- *223. *B. hordeaceus* L. Rare; introduced from Europe.
- *224. *B. hordeaceus glabrescens* Shear. An infrequent introduction. (Europe).
- *225. *B. racemosus* L. (*B. commutatus* Schrad.) Infrequent. (Europe).
- 226. *B. ciliatus* L. A native species common in woods.
- *227. *B. japonicus* Thunb. A pretty species introduced from Japan via Europe. It has often been referred to *B. arvensis* of Gray's Manual not of L. (Japan).
- *228. *B. tectorum* L. A weedy species becoming very common in dry waste places. (Europe).
- 229. *B. purgans* L. A common native woodland species.
- *230. *B. inermis* Leyss. The cultivated Brome Grass. Frequently escaped. (Europe).
- 231. *B. altissimus* Pursh. Frequent in woods.
- 232. *B. Kalmii* Gray. Infrequent in woods.
- *233. *B. marginatus* Nees. (*B. breviaristatus* Buckley). Cultivated and occasionally escaped. (Western U. S.)

Lolium L.

- *234. *L. perenne* L. Common Darnel or Rye Grass. Cultivated and escaped. (Eurasia).
- *235. *L. multiflorum* Lam. (*L. italicum* R. Br.) Rare as an escape. (Europe).
- *236. *L. temulentum* L. Bearded Darnel. A frequent weed in grain fields and waste places. (Eurasia).

Agropyron Gaertn.

- 237. *A. Smithii* Rydb. A common and valuable native species.
- *238. *A. repens* (L.) Beauv. Quack Grass. A common, introduced species, and very difficult to eradicate. (Europe).
- 239. *A. biflorum* (Brignoli) R. & S. (*A. violaceum* Lange.) Rare in dry ground; Winneshiek and Dickinson counties.

- 240. *A. tenerum* Vasey. Slender Wheat Grass. Common in dry ground.
- 241. *A. caninoides* (Rameley) Beal. (*A. caninum* of most American authors) Rare in the northern part.
- 242. *A. Richardsonii* Schrad. Frequent in dry ground.

Hordeum (Tourn.) L. Barley.

- *243. *H. jubatum* L. Squirrel Tail, Wild Barley. A noxious weed in pastures and waste places. Native for northeast. (N. Am. and Eurasia).
- 244. *H. pusillum* Nutt. Frequent.
- 245. *H. Pammelii* Scribn. & Ball. The type from Humboldt Co. It resembles the cultivated species.
- *246. *H. vulgare* L. The common cultivated species. Occasionally adventive along highways. (Eurasia).

Secale (Tourn.) L. Rye.

- *247. *S. cereale* L. Cultivated and adventive along highways. (Eurasia).

Triticum L. Wheat.

- *248. *T. vulgare* L. Occasionally adventive along highways. (Eurasia).

Elymus L. Wild Rye.

- 249. *E. virginicus* L. Common, especially in open woods.
- 250. *E. virginicus submuticus* Hook. (*E. curvatus* Piper). Frequent in open woods, or near timber.
- 251. *E. hirsutiglumis* Scribn. (*E. virginicus* var. Hitch.) Rare.
- 252. *E. canadensis* L. Common and extremely variable.
- 253. *E. glabriflorus* (Vasey) Scribn. & Ball. Frequent in shady woods in Lee Co. (Jess L. Fuels).
- 254. *E. robustus* Scribn. & J. G. Smith. Common; resembles *E. canadensis*, but with large, wider spikes and wide-spreading awns.
- 255. *E. robustus vestitus* Wiegand. Frequent. The glumes pubescent.
- 256. *E. brachystachys* Scribn. & Ball. Frequent.
- 257. *E. striatus* Willd. Common in or near timber. Forms which have been referred to the varieties *arkansana*, *Ballii* and *villosus* also occur sparingly.
- 258. *E. Macounii* Vasey. A rare species with long, slender spikes. Infrequent in our northwestern section.
- 259. *E. diversiglumis* Scribn. & Ball. Rare in the northern part.

Hystrix Moench.

- 260. *H. patula* Moench. Bottle-brush Grass. Very common in open woods.

18. CYPERACEAE (*Sedge Family*)

Cyperus (Tourn.) L. Galingale.

- 261. *C. diandrus* Torr. Common; sandy banks of lakes and streams.
- 262. *C. rivularis* Kunth. Common in low ground.
- 263. *C. inflexus* Muhl. (*C. aristatus* Rottb.) Common; sandy shores of lakes and streams.
- 264. *C. Schweinitzii* Torr. Frequent on sandy banks.

- 265. *C. acuminatus* Torr. & Hook. Rare in the southeastern part.
- 266. *C. esculentus* L. Nut Grass. Common; a weedy species in cultivated land.
- 267. *C. erythrorhizos* Muhl. Frequent on sandy shores.
- 268. *C. speciosus* Vahl. Common in marshes; now considered distinct from *C. ferax* Rich. of the s.e. United States.
- 269. *C. filiculmis* Vahl. Frequent in the eastern part.

Dulichium Pers.

- 270. *D. arundinaceum* (L.) Britton. Rather rare; low, wet situations; eastern and southern.

Eleocharis R. Br. Spike Rush.

- 271. *E. obtusa* (Willd.) Schultes. Infrequent in the eastern part.
- 272. *E. palustris* (L.) R. & S. Common in shallow water.
- 273. *E. glaucescens* Willd. (*E. palustris* var. Gray). Very common in low, wet ground.
- 274. *E. acicularis* (L.) R. & S. Very common in low, wet ground.
- 275. *E. Wolffi* A. Gray. Very rare on low prairies; Emmet Co. (R. I. Cratty).
- 276. *E. tenuis* (Willd.) Schultes. Rare; resembling the next, but the root perennial.
- 277. *E. intermedia* (Muhl.) Schultes. Rare; an annual species.
- 278. *E. acuminata* (Muhl.) Nees. Frequent; eastern and southern.

Stenophyllus Raf.

- 279. *S. capillaris* (L.) Britton. (*Fimbristylis* Gray) Frequent in the southeast.

Fimbristylis Vahl.

- 280. *F. autumnalis* (L.) R. & S. Rare in the southeastern part.

Scirpus (Tourn.) L. Bulrush, Club-rush.

- 281. *S. americanus* Pers. (*S. pungens* Vahl.) Frequent.
- 282. *S. validus* Vahl. (not *S. lacustris* L. of the Old World which has a three-cleft style) Our common Bulrush in marshes, and shallow water.
- 283. *S. occidentalis* (S. Wats.) Chase. Infrequent in shallow water; Emmet Co. (B. O. Wolden).
- 284. *S. heterochaetus* Chase. Rare; Emmet Co. (B. O. Wolden) The last two species perhaps overlooked because of their close resemblance to *S. validus*.
- 285. *S. fluviatilis* Gray. A coarse species growing along streams and in marshes.
- 286. *S. atrovirens* Muhl. Very common in wet soil.
- 287. *S. lineatus* Michx. Frequent, eastern and southern.
- 288. *S. cyperinus* (L.) Kunth. The typical form infrequent.
- 289. *S. cyperinus pelius* Fernald. Frequent in the eastern part.
- 290. *S. pedicellatus* Fernald. Infrequent; Chickasaw Co. (W. D. Spiker) The last three not easily distinguished.

Eriophorum L. Cotton Grass.

- 291. *E. tenellum* Nutt. (*E. gracile* Koch.; *E. gracile paucinervium* Engelm.) Rare in our northern bogs; Emmet Co. (R. I. Cratty).
- 292. *E. angustifolium* Roth. (*E. polystachyon* of authors in part). Frequent in cold northern bogs.

Hemicarpha Nees & Arn.

- 293. *H. micrantha* (Wahl.) Britton. (*H. subsquarrosa* Nees.) Rare; central and eastern.

Rhynchospora Vahl. Beak Rush.

294. *R. capillacea* Torr. Rare in marshes; Emmet Co. (B. O. Wolden).

Scleria Bergius. Nut Rush.

295. *S. triglomerata* Michx. Rare in the eastern part.

Carex L. Sedge

296. *C. chordorrhiza* Ehrh. Rare in cold bogs; Emmet Co. (R. I. Cratty, F. W. Paige).
 297. *C. Eleocharis* Bailey. (*C. stenophylla* of Am. authors not of Wahl.) Very rare in dry ground; Emmet Co. (R. I. Cratty) Lyon Co. (B. Shimek).
 298. *C. Sartwellii* Dewey. Frequent in marshes.
 299. *C. convoluta* Mackenzie (*C. rosea* of Gray's Manual 7th ed.) Common in rich woods.
 300. *C. rosea* Schkuhr. (*C. rosea radiata* of Gray's Manual) Infrequent; a delicate species.
 301. *C. plana* Mackenzie (*C. Muhlenbergii enervis* Boott) Rare; Johnson Co. (M. P. Somes).
 302. *C. cephalophora* Muhl. Infrequent.
 303. *C. Leavenworthii* Dewey. Infrequent.
 304. *C. gravida* Bailey. Common in open woods, and occasionally on prairies.
 305. *C. cephaloidea* Dew. Rare.
 306. *C. sparganioides* Muhl. Frequent in woods; central and southern.
 307. *C. alopecoidea* Tuck. Infrequent; Emmet Co. (R. I. Cratty, B. O. Wolden)
 308. *C. conjuncta* Boott. Rare; southeastern.
 309. *C. vulpinoidea* Michx. Our commonest species in low ground.
 310. *C. brachyglossa* Mackenzie (*C. xanthocarpa* Bicknell) Frequent in the southeastern part.
 311. *C. diandra* Schrank. (*C. teretiuscula* Good.) Frequent in northern peat bogs.
 312. *C. prairea* Dewey. (*C. teretiuscula* var. Britton; *C. diandra ramosa* Fernald) Rare in marshy ground; Emmet Co. (R. I. Cratty)
 313. *C. stipata* Muhl. Common in open woods.
 314. *C. crus-corvi* Shuttlew. Infrequent.
 315. *C. Deweyana* Schwein. Rare; Webster and Dickinson counties.
 316. *C. interior* Bailey. (*C. scirpoides* Schkuhr.) Frequent on low prairies.
 317. *C. synchnocephala* Carey. Rare; edges of swamps; Emmet and Dickinson counties.
 318. *C. scoparia* Schkuhr. Frequent in dry soil.
 319. *C. tribuloides* Wahl. Frequent in low woods.
 320. *C. cristatella* Britton. (*C. cristata* Schwein., *C. tribuloides* var. Bailey) Frequent.
 321. *C. projecta* Mackenzie. (*C. tribuloides reducta* Bailey) Frequent in woods.
 322. *C. muskingumensis* Schwein. Rare.
 323. *C. Bebbii* Olney. Frequent in low ground.

324. *C. normalis* Mackenzie. (*C. mirabilis* Dewey). Infrequent.
325. *C. tenera* Dewey. Rare. (*C. straminea* in part.)
326. *C. festucacea* Schkuhr. Rare in the southeastern part.
327. *C. brevior* (Dewey) Mackenzie. (*C. festucacea* var. Fernald) Common.
328. *C. Bicknellii* Britton. Frequent on upland prairie.
329. *C. suberecta* (Olney) Britton. Infrequent.
330. *C. Jamesii* Schwein. Rather rare in low woods.
331. *C. communis* Bailey (*C. pedicellata* Britton) Infrequent.
332. *C. pennsylvanica* Lam. Very common on upland prairies.
333. *C. varia* Muhl. Infrequent.
334. *C. hirtifolia* Mackenzie (*C. pubescens* of Gray's Manual) Rare (Holway).
335. *C. pedunculata* Muhl. Infrequent in the southeast.
336. *C. Richardsoni* R. Br. Rare; Poweshiek Co. (M. E. Jones).
337. *C. eburnea* Boott. (*C. setifolia* Britton) Frequent on dry wooded hills.
338. *C. Meadii* Dewey. Common on prairies.
339. *C. tetanica* Schkuhr. Rare; Emmet Co. (B. O. Wolden).
340. *C. laxiculmis* copulata (Bailey) Fernald. Rare; Muscatine Co. (Barnes & Miller).
341. *C. albusina* Sheldon. (*C. laxiflora latifolia* Boott). Common in woods.
342. *C. laxiflora* Lam. Infrequent and variable.
343. *C. blanda* Dewey (*C. laxiflora* var. Gray) A woodland species; frequent and extremely variable.
344. *C. Shriveri* Dewey. (*C. granularis* var. Britton) Rare; Webster Co. (O. M. Oleson).
345. *C. granularis* Muhl. Rare; Johnson Co. (B. Shimek).
346. *C. Crawei* Dewey. Rare on low prairies.
347. *C. oligocarpa* Schkuhr. Rare in open woods.
348. *C. Hitchcockiana* Dewey. Rare; Jasper Co. (H. W. Norris).
349. *C. conoidea* Schkuhr. Rare.
350. *C. grisea* Wahl. Frequent in woods.
351. *C. gracillima* Schwein. Rare in woods; Emmet Co. (B. O. Wolden).
352. *C. Davisii* Schwein. & Torr. Frequent in low woods in the southern part.
353. *C. assiniboinensis* Boott. Rare; Emmet Co. (B. O. Wolden).
354. *C. Sprengelii* Dewey. (*C. longirostris* Torr.) Common in woods.
355. *C. hirsutella* Mackenzie (*C. triceps hirsuta* Bailey). Rare.
356. *C. limosa* L. Frequent in northern marshes.
357. *C. Buxbaumii* Wahl. (*C. fusca* Bailey, *C. polygama* Schkuhr.) Frequent in bogs and very wet places.
358. *C. Shortiana* Dewey. Frequent in woods.
359. *C. stricta* Lam. Frequent in very wet places.
360. *C. strictior* Dewey. (*C. stricta angustata* of Gray's Manual). Frequent.
361. *C. Haydeni* Dewey. (*C. stricta decora* Bailey) Frequent in wet places.
362. *C. aquatilis* Wahl. Rare; Emmet Co. (B. O. Wolden).

- 363. *C. Emoryi* Dewey. Rare; Fayette Co. (Bruce Fink).
- 364. *C. lacustris* Willd. (*C. riparia* Muhl. not Curtis) Frequent in marshes.
- 365. *C. lanuginosa* Michx. Frequent on low prairies.
- 363. *C. lasiocarpa* Ehrh. (*C. filiformis* Good. Not L.) Frequent in northern marshes.
- 367. *C. trichocarpa* Muhl. Infrequent.
- 368. *C. laeviconica* Dewey. (*C. trichocarpa Deweyi* Bailey) Rare; southeastern section.
- 369. *C. atherodes* Spreng. (*C. aristata* R. Br.) Frequent in marshes.
- 370. *C. vesicaria monile* (Tuck.) Fernald. (*C. monile* Tuck.) Infrequent in wet places. A beautiful species.
- 371. *C. rostrata* Stokes. Rare in wet situations. Emmet Co. (B. O. Wolden).
- 372. *C. Tuckermanni* Dewey. Very rare; Jasper Co. (H. W. Norris).
- 373. *C. retrorsa* Schwein. Frequent in rich woods.
- 374. *C. hystericina* Muhl. Frequent in marshy places.
- 375. *C. comosa* Boott. Infrequent; edge of swamps.
- 376. *C. squarrosa* L. Rare; Appanoose Co. (T. J. Fitzpatrick).
- 377. *C. typhina* Michx. (*C. typhinoides* Schwein.) Rare in low woods.
- 378. *C. intumescens* Rudge. Infrequent.
- 379. *C. Asa-Grayi* Bailey. Infrequent.
- 380. *C. lupulina* Muhl. Frequent in low woods.
- 381. *C. lupuliformis* Sartw. Infrequent. Lee and Decatur counties.

20. ARACEAE (*Arum Family*)

Arisaema Mart.

- 382. *A. triphyllum* (L.) Schott. Indian Turnip, Jack-in-the-pulpit. Common in rich woods.
- 383. *A. Dracontium* (L.) Schott. Green Dragon. Infrequent in similar situations.

Symplocarpus Salisb.

- 384. *S. foetidus* (L.) Nutt. (*Spathyema* Raf.) Skunk cabbage. An ill-smelling plant, quite rare in swampy woods.

Acorus L.

- 385. *A. Calamus* L. Sweet Flag, Calamus. Common in swampy places, especially in the northern part.

22. LEMNACEAE (*Duckweed Family*)

Spirodela Schleid.

- 386. *S. polyrhiza* (L.) Schleid. Common. Floating on still water.

Lemna L. Duckweed.

- 387. *L. trisulca* L. Very common on stagnant water.
- 388. *L. minor* L. Infrequent with the above.

Wolffia Horkel.

- 389. *W. punctata* Griseb. (*W. braziliensis* of authors not of Weddell) Infrequent. Floating as little grains on still water. Our smallest flowering plant.

31. COMMELINACEAE (*Spiderwort Family*)**Tradescantia** (Rupp.) L. Spiderwort.390. *T. reflexa* Raf. Frequent.391. *T. bracteata* Small. Our commonest species; formerly confused with *T. virginiana* L.392. *T. occidentalis* (Britton) Smyth. Infrequent.**Commelina** (Plum.) L.*393. *C. communis* L. Day Flower. An occasional escape. (Asia).394. *C. virginica* L. Rare near the southern border. Lee Co. (Jess L. Fults)32. PONTEDERIACEAE (*Pickereel-weed Family*)**Pontederia** L.395. *P. cordata* L. Pickereel-weed. Infrequent; eastern and southern.**Heteranthera** R. & P. Mud Plantain.396. *H. limosa* (Swartz) Willd. Rare; Fremont Co. (A. S. Hitchcock).397. *H. dubia* (Jacq.) MacM. (*H. graminea* Vahl.) Infrequent in mud or shallow water.34. JUNCACEAE (*Rush Family*)**Juncus** (Tourn.) L. Rush.398. *J. bufonius* L. Infrequent on sandy banks.399. *J. tenuis* Willd. (*J. bicornis* Michx.) Our commonest species in wet soil.400. *J. secundus* Beauv. A form nearest this. Rare; Lee Co. (Jess L. Fults).401. *J. confusus* Coville. Rare, Blackhawk Co. (L. H. Pammel). Det. by Dr. Rydberg.402. *J. Dudleyi* Wiegand. Rare; Emmet Co. (B. O. Wolden).403. *J. littorum* Rydb. (*J. balticus littoralis* Engelm. not *J. littoralis* C. A. Meyer) Infrequent in low, acid soil.404. *J. effusus* L. A common bog species of very wide distribution.405. *J. canadensis* J. Gay. Infrequent.406. *J. acuminatus* Michx. A slender, knotted-leaved species; infrequent in low places.407. *J. nodosus* L. Frequent in low places.408. *J. Torreyi* Coville. A common species, resembling the preceding, but much larger.**Luzula** DC. Wood Rush.409. *L. campestris multiflora* (Ehrh.) Celak. (*J. campestris* of Am. authors; *Juncoides intermedium* Rydb.) Frequent in rich woods.410. *L. carolinae* S. Wats. (*L. saltuensis* Fernald; *Juncoides carolinae* Kuntze) Rare; Delaware Co. (L. H. Pammel).36. LILIACEAE (*Lily Family*)**Zygadenus** Michx.411. *Z. chloranthus* Richards. (*Z. elegans* of authors not Pursh). Zygadene. Common on our northern prairies.**Melanthium** L.412. *M. virginicum* L. Bunch Flower. Frequent in the southern half.**Veratrum** (Tourn.) L.413. *V. Woodii* Robbins. False Hellebore. Rare in rich woods.

Uvularia L.

414. *U. grandiflora* J. E. Smith. Bellwort. Common in rich woods.

Oakesiella Greene. (*Oakesia* S. Wats. not Tuck.)

415. *O. sessilifolia* (L.) Greene. (*Oakesia* S. Wats.) Rare.

Allium (Tourn.) L. Onion.

416. *A. tricoccum* Ait. Frequent in woods.
 417. *A. stellatum* Ker. Common in rather dry soil.
 418. *A. canadense* L. Frequent on low prairies.
 419. *A. mutabile* Michx. Very rare; Lee Co. (Jess L. Fufts) A southern species barely entering our limits.

Nothoscordum Kunth.

420. *N. bivalve* (L.) Britton. (*Allium striatum* Jacq.) Rare.

Hemerocallis L.

- *421. *H. fulva* L. Common Day Lily. A frequent escape from cultivation, especially in the southeastern part. (Asia.)

Lilium (Tourn.) L. Lily.

422. *L. philadelphicum andinum* (Nutt.) Ker. (*L. umbellatum* Pursh) Common and variable.
 423. *L. michiganense* Farwell. Frequent. Formerly referred to *L. canadense* and *L. superbum* which do not range this far west.

Erythronium Dog's-tooth Violet.

424. *E. albidum* Nutt. Very common in rich woods.
 425. *E. americanum* Ker. Yellow Adder's Tongue. Infrequent.
 426. *E. mesochoreum* Knerr. Rare in the western part.

Camassia Lindl. (*Quamassia* Raf.)

427. *C. esculenta* (Ker.) Robinson. (*Quamassia hyacinthina* Britton) Eastern Camas or Wild Hyacinth. Infrequent in or near timber.

Yucca (Rupp.) L.

428. *Y. glauca* Nutt. Bear Grass or Spanish Bayonet. Rare in the northwestern part in dry soil.

Asparagus (Tourn.) L.

- *429. *A. officinalis* L. Common Asparagus. A frequent escape, the seeds carried by birds. (Europe.)

Smilacina Desf.

430. *S. racemosa* (L.) Desf. False Spikenard. Very common in woods.
 431. *S. stellata* (L.) Desf. Common; a much smaller plant.

Maianthemum Wiggers.

432. *M. canadense* Desf. (*Unifolium* Greene) Infrequent in woods.

Polygonatum (Tourn.) Hill.

433. *P. commutatum* (R. & S.) Dietr. Solomon's Seal. Very common in rich woods.

Trillium L. Wake Robin.

434. *T. recurvatum* Beck. Frequent; often confused with *T. sessile*.
 435. *T. erectum* L. Frequent.
 436. *T. grandiflorum* (Michx.) Salisb. Frequent northeast.
 437. *T. cernuum* L. Infrequent.
 438. *T. declinatum* (Gray) Gleason. Common in rich woods.
 439. *T. nivale* Riddell. Frequent; one of our earliest spring flowers.

Smilax (Tourn.) L. Green Brier.

440. *S. herbacea* L. Carrion Flower. Our commonest herbaceous species.

- 441. *S. ecirrhata* (Engelm.) S. Wats. Rare; tendrils lacking, or only the upper petioles tendril bearing.
- 442. *S. lasioneuron* Hook. Frequent. The fruit clusters very large, bearing 75 to 100 or more berries.
- 443. *S. rotundifolia* L. Frequent in moist thickets; the stem perennial.
- 444. *S. hispida* Muhl. Frequent in woods; the hispid stem perennial.
- 445. *S. Pseudo-china* L. Infrequent; a glabrous, tuberous-rooted species. Further observation needed.

38. AMARYLLIDACEAE (*Amaryllis Family*)

Hypoxis L.

- 446. *H. hirsuta* (L.) Coville. Star Grass. Very common on low prairies.

41. DIOSCOREACEAE (*Yam Family*)

Dioscorea (Plumier) L.

- 447. *D. villosa* L. (*D. paniculata* Michx.) Wild Yam. Frequent in woods, especially in the southern part.

42. IRIDACEAE (*Iris Family*)

Iris (Tourn.) L.

- 448. *I. versicolor* L. Blue Flag. Iris. Common in marshy places.

Belamcanda Adans.

- *449. *B. chinensis* (L.) DC. Blackberry Lily. Rare as an escape along highways. (Asia).

Sisyrinchium L. Blue-eyed Grass.

- 450. *S. campestre* Bicknell. Very common on low prairies.
- 451. *S. mucronatum* Michx. Rare; Cedar Co. (A. S. Hitchcock).
- 452. *S. graminoides* Bicknell. Infrequent in the southern part.

48. ORCHIDACEAE (*Orchis Family*)

Cypripedium L. Lady's Slipper, Moccasin Flower.

- 453. *C. parviflorum pubescens* (Willd.) Knight. Frequent in or near timber.
- 454. *C. candidum* Muhl. Once common on low prairies, now becoming rare.
- 455. *C. hirsutum* Mill. (*C. reginae* Walt., *C. spectabile* Salisb.) Rich woods; becoming rare.

Orchis (Tourn.) L. Showy Orchis.

- 456. *O. spectabilis* L. (*Galeorchis* Rydb.) Rare in rich woods.

Habenaria Willd. Rein Orchis.

- 457. *H. bracteata* (Willd.) R. Br. (*Coeloglossum* Farwell) Infrequent.
- 458. *H. hyperborea* (L.) R. Br. (*Limnorchis* Rydb.) Infrequent.
- 459. *H. clavellata* (Michx.) Spreng. (*Denslovina* Rydb.) Quite rare; Fayette and Muscatine counties.
- 460. *H. Hookeri* Gray. (*Lysias Hookerianum* Rydb.) Infrequent in the eastern part.
- 461. *H. leucophaea* (Nutt.) Gray. (*Blephariglottis* Farwell). More common in the northern part.
- 462. *H. psychodes* (L.) Sw. (*Blephariglottis* Rydb.) Infrequent.

Pogonia Juss.

463. *P. trianthophora* (Sw.) BSP. (*P. pendula* Lindl.) Rare in the northeast.

Calopogon R. Br.

464. *C. pulchellus* (Sw.) R. Br. Rare in the eastern part.

Spiranthes Richard. Ladies' Tresses.

465. *S. gracilis* (Bigelow) Beck. (*Ibidium* House) Infrequent.
 466. *S. cernua* (L.) Richard. (*Ibidium* House) Infrequent on low prairies.
 467. *S. Romanzoffiana* Cham. (*Gyrostachys* Rydb., *Ibidium strictum* House) Rare in bogs. Emmet Co. (B. O. Wolden).

Epipactis (Haller) Boehm.

468. *E. pubescens* (Willd.) A. A. Eaton. (*Goodyera* R. Br., *Pera-mium* MacM.) Rattle-snake Plantain. Rare; Winneshiek Co.

Corallorrhiza (Haller) R. Br. Coral Root.

469. *C. odontorrhiza* Nutt. (*C. Corallorrhiza* Karst.) Rare; Johnson Co. (T. J. Fitzpatrick).

Microstylis (Nutt.) Eaton.

470. *M. unifolia* (Michx.) BSP. (*Malaxis* Michx.) Green Adder's Mouth. Rare in the northeastern part.

Liparis Richard. Twayblade.

471. *L. liliifolia* (L.) Richard. Infrequent in moist woods.
 472. *L. Loeselii* (L.) Richard. Very rare; moist, shady places.

Aplectrum (Nutt.) Torr.

473. *A. hyemale* (Muhl.) Torr. Adam-and-Eve, Putty Root. Rare; Johnson Co. (T. J. Fitzpatrick).

54. SALICACEAE (*Willow Family*)**Populus** (Tourn.) L. Poplar, Aspen.

- *474. *P. alba* L. White or Silver Poplar. A frequent escape to roadsides, mostly by suckering. (Europe).
 475. *P. tremuloides* Michx. American Aspen, Quaking Aspen. Common.
 476. *P. grandidentata* Michx. Large-toothed Aspen. Frequent.
 *477. *P. balsamifera* L. (*P. Tacamahaca* Miller) Balsam Poplar or Tacamahac. An occasional escape. Our only Iowa specimen is from Emmet Co. (B. O. Wolden). Native farther north and east.
 *478. *P. canadensis* Ait. Balm of Gilead. Frequent in cultivation and escaped. (Europe).
 *479. *P. deltoides* Marsh. The eastern Cottonwood; occasional in cultivation and as an escape. Now considered distinct from the next. (Eastern U. S.).
 480. *P. Sargentii* Dode. Cottonwood. Frequent as a native, and common in cultivation and as an escape.

Salix (Tourn.) L. Willow

481. *S. nigra* Marsh. Black Willow. Common, especially along streams.
 482. *S. amygdaloides* Anders. Peach-leaved Willow. Common along streams.

483. *S. lucida* Muhl. Shining Willow. Quite rare in the northern half. A beautiful species.
- *484. *S. fragilis* L. Crack Willow. Our commonest willow in cultivation and a frequent escape. The twigs are very brittle. (Europe).
- *485. *S. alba* L. White Willow. Cultivated and escaped. Less common than the preceding. (Europe).
486. *S. interior* Rowlee. (*S. longifolia* of Am. authors) Sand-bar Willow. A narrow-leaved species, very common, especially near streams.
487. *S. cordata* Muhl. Heart-leaved Willow. Frequent and variable.
488. *S. missouriensis* Bebb. Common, but scarcely distinct from the preceding.
489. *S. pedicellaris* Pursh. Rare in our northern marshes. Formerly referred to the European *S. myrtilloides*.
490. *S. discolor* Muhl. Pussy Willow. Common. Our earliest flowering species.
491. *S. eriocephala* Michx. (*S. discolor* var. Robs. & Fernald) Frequent.
492. *S. petiolaris* Smith. Infrequent in marshy places in the northern half.
493. *S. subsericea* (Anders.) Schneider. Rare in a bog near the Rock Island R.R. gravel pit, Graettinger, Emmet Co. (B. O. Wolden) Dr. Rydberg considered this a hybrid—*S. sericea* x *petiolaris*.
494. *S. humilis* Marsh. Frequent on prairies, and in open woods.
495. *S. tristis* Ait. Dwarf Gray Willow. Rare in the eastern part.
496. *S. sericea* Marsh. Silky Willow. Rare; Henry and Fayette counties.
497. *S. Bebbiana* Sargent. (*S. rostrata* of Am. authors) Frequent, especially in the northern half.
498. *S. candida* Fluegge. Sage or Hoary Willow. Rare in our northern marshes.

58. JUGLANDACEAE (*Walnut Family*)**Juglans** L.

499. *J. cinerea* L. Butternut. Frequent.
500. *J. nigra* L. Black Walnut. More common than the preceding, and one of our most valuable native trees.

Carya Nutt. (*Hicoria* Raf.) Hickory.

501. *C. Pecan* (Marsh.) Britton. (*C. illinoensis* K. Koch., *Hicoria Pecan* Britton) Frequent in the southeastern part.
502. *C. ovata* (Mill.) K. Koch. (*Hicoria* Britton) Shell-bark or Shag-bark Hickory. Common.
503. *C. luciniosa* (Michx. fil.) Louden. (*Hicoria* Sargent) King Nut. Frequent in the southeast.
504. *C. alba* (L.) K. Koch. (*Hicoria* Britton) Mocker-nut Hickory. Southern and eastern part. Not very common.
505. *C. glabra* (Mill.) Spach. (*Hicoria* Britton) Pig-nut Hickory. Rare in the southeastern part. (L. H. Pammel).
506. *C. cordiformis* (Wang.) K. Koch. (*Hicoria* Britton) Bitter-nut. Very common.

59. BETULACEAE (*Birch Family*)**Corylus** (Tourn.) L.

507. *C. americana* Walt. Common Hazel-nut. Our commonest species.
 508. *C. rostrata* Ait. (*C. cornuta* Marsh.) Beaked Hazel-nut. Frequent in the northeast.

Ostrya (Michx.) Seop.

509. *O. virginiana* (Mill.) K. Koch. Ironwood or Hop Hornbeam. Very common.

Carpinus (Tourn.) L.

510. *C. caroliniana* Walt. Blue Beech, American Hornbeam. Frequent.

Betula (Tourn.) L.

511. *B. lutea* Michx. fil. Gray or Yellow Birch. Frequent in the eastern part.
 512. *B. lenta* L. Cherry or Sweet Birch. Very rare in Iowa. Our only specimen from Lee Co. (Jess L. Fufts).
 513. *B. nigra* L. River or Red Birch. Frequent in the eastern half.
 514. *B. papyrifera* Marsh. (*B. alba* var. Spach) Paper, Canoe or White Birch. Frequent in the northeastern part; also cultivated.
 *515. *B. pendula* Roth. (*B. alba* L. in part) European White Birch, common in cultivation; rare as an escape; Emmet Co. (B. O. Wolden).
 516. *B. pumila glandulifera* Regel. Rare; Chickasaw and Allamakee counties.

Alnus (Tourn.) Hill.

517. *A. incana* (L.) Moench. Alder. Most frequent in the eastern half.

60. FAGACEAE (*Beech Family*)**Quercus** (Tourn.) L. Oak

518. *Q. alba* L. White Oak. Common except in the western part; one of our most valuable trees.
 519. *Q. stellata* Wang. Iron or post oak. Infrequent in the southern part.
 520. *Q. lyrata* Walt. Swamp Oak. Rare; only a few trees known—one in Johnson Co. near Amana (Shimek), one in Lee Co. near Keokuk (Pammel) and several in Appanoose Co. near Centerville (Trenk).
 521. *Q. bicolor* Willd. Swamp White Oak. Frequent in the eastern part; the acorns long-peduncled.
 522. *Q. macrocarpa* Michx. Bur Oak. Our commonest species throughout the state.
 523. *Q. Muhlenbergii* Engelm. (*Q. acuminata* Houba) Yellow or chestnut oak. Frequent; central, eastern and southern sections.
 524. *Q. prinoides* Willd. Shrubby chestnut Oak. Rare; Madison and Decatur counties.
 525. *Q. marina* (Marsh.) Ashe. (*Q. rubra*) of most Am. authors not of L.) Red Oak. Very common and variable.
 526. *Q. palustris* Moench. Swamp Oak. Frequent; southern and eastern sections.

527. *Q. ellipsoidalis* E. J. Hill. Black Oak. A common and extremely variable species.
 528. *Q. velutina* Lam. Quercitron Oak. Frequent; a beautiful but variable species.
 529. *Q. Schneekii* Britton. Rare; a single specimen from near Black Springs, Johnson Co. (H. P. Hagge.)
 530. *Q. marilandica* Muench. Black Jack Oak. Infrequent; eastern and southern.
 531. *Q. imbricaria* Michx. Shingle Oak. A handsome species, frequent in the east and southern sections.

The oaks contain many perplexing forms, especially in the *ellipsoidalis* group, some of which approach very closely the northeastern *Q. borealis* Michx. There are also represented in the herbarium several well-marked hybrids. Among these are *Q. velutina* x *marilandica*, *Q. bicolor* x *macrocarpa*, *Q. velutina* x *ellipsoidalis*, and *Q. imbricaria* x *velutina*.

61. ULMACEAE (*Elm Family*)

Ulmus (Tourn.) L.

532. *U. fulva* L. Slippery or Red Elm. Common.
 533. *U. americana* L. American or White Elm. Very common, and much used for street planting.
 534. *U. Thomasii* Sargent. (*U. racemosa* Thomas) Cork or Rock Elm. Frequent.

Celtis (Tourn.) L.

535. *C. occidentalis* L. Hackberry. Common.
 536. *C. occidentalis crassifolia* (Lam.) Gray. Rough-leaved Hackberry. Frequent.

62. MORACEAE (*Mulberry Family*)

Cannabis (Tourn.) L.

- *537. *C. sativa* L. Common Hemp. Frequent in woods and around dwellings; the seeds used in food for canary birds. (S. Asia).

Humulus L.

538. *H. Lupulus* L. Common Hop. Infrequent, border of woods.

Maclura Nutt. (*Toxylon* Raf.)

- *539. *M. pomifera* (Raf.) Schneider. Osage Orange. Formerly much used for hedge fences, and escaped in the southern part. (S. United States).

Morus (Tourn.) L.

540. *M. rubra* L. Red Mulberry. Frequent except in the northwestern part.
 *541. *M. alba* L. White Mulberry. A common escape from cultivation. (Europe).

63. URTICACEAE (*Nettle Family*)

Urtica (Tourn.) L.

542. *U. procera* Muhl. (*U. gracilis* of Am. authors not of Ait.) Common Wood Nettle. Frequent in timber and hedge rows.
 *543. *U. urens* L. Small or Dwarf Nettle. Introduced around dwellings and in waste places. (Europe).

Laportea Gaud.

544. *L. canadensis* (L.) Gaud. (*Urticastrum divaricatum* Kuntze)
Wood or Canada Nettle. Common in rich woods.

Pilea Lindl.

545. *P. pumila* (L.) Gray (*Adicea* Raf.) Rich or Clear Weed. Common in low, rich woods.

Boehmeria Jacq.

546. *B. cylindrica* (L.) Sw. False Nettle. Infrequent in moist, shady woods.

Parietaria (Tourn.) L.

547. *P. pennsylvanica* Muhl. Pellitory. Common with the three preceding species.

67. SANTALACEAE (*Sandalwood Family*)**Comandra** Nutt.

548. *C. umbellata* (L.) Nutt. Bastard Toad-flax. Common on upland prairies and in dry open woods. The roots often parasitic.

72. ARISTOLOCHIACEAE (*Birthwort Family*)**Asarum** (Tourn.) L. Wild Ginger.

549. *A. canadense* L. Frequent in low woods.
550. *A. canadense acuminatum* Ashe. Our commonest form.
551. *A. canadense reflexum* (Bicknell) Robinson. Infrequent. All these are quite variable.

75. POLYGONACEAE (*Buckwheat Family*)**Rumex** L. Dock.

- *552. *R. Patientia* L. Infrequent around German settlements. The leaves used for greens. (Europe).
553. *R. Britannica* L. Great Water Dock. Common in swampy places.
*554. *R. crispus* L. Yellow Dock. A very common, unlawful weed in fields and waste places. (Europe).
555. *R. mexicanus* Meisn. Common; often confused with the next; all three perianth lobes tubercled.
556. *R. altissimus* Wood. Smooth or Pale Dock. Common in alluvial soil; only one perianth lobe tubercled.
557. *R. verticillatus* L. Frequent in swamps.
*558. *R. obtusifolius* L. Bitter Dock. Infrequent. Naturalized from Europe.
559. *R. persicarioides* L. (*R. maritimus* of older Manuals) Common in marshes.
*560. *R. Acetosella* L. Sheep Sorrel. A common noxious perennial weed. (Europe).

Polygonum (Tourn.) L. Knotweed.

561. *P. exsertum* Small. Rare; Chickasaw and Union counties.
562. *P. aviculare* L. Knot-grass. A very common dooryard weed.
563. *P. neglectum* Besser. (*P. aviculare angustissimum* Meisn.) Infrequent; an upright, narrow-leaved form.
564. *P. erectum* L. A common, upright plant, resembling *P. aviculare*, but of stockier growth, and larger, and wider leaves.

565. *P. ramosissimum* Michx. A tall plant, common in rather dry ground.
566. *P. tenue* Michx. A low, narrow-leaved species, frequent in dry ground.
567. *P. lapathifolium* L. (*Persicaria* S. F. Gay) Very common in low, waste places.
568. *P. amphibium* L. (*Persicaria* S. F. Gay) Common in still water; the large, shiny leaves floating.
569. *P. amphibium Hartwrightii* (Gray) Bissel. Frequent; an upright, pubescent plant, in shallow water or wet soil.
570. *P. Muhlenbergii* (Meisn.) S. Wats. (*Persicaria* Small, *P. coccinea* Greene) Tan-weed, Devil's Shoe-string. A common, troublesome weed in low, cultivated soil.
571. *P. iowense* (Rydb.) Cratty, n. comb. (*Persicaria* Rydb.) Resembling the preceding, but has wider leaves, and is very pubescent throughout. Collected near Ames, July 29, 1902, by R. E. Buchanan. Type in I.S.C. Herbarium, No. 77, 688, Co-type in N. Y. Botanic Garden.
572. *P. pennsylvanicum* L. (*Persicaria* Small) A very common, annual species.
573. *P. longistylum* Small. (*Persicaria* Small) Very rare; $3\frac{1}{2}$ miles s.w. of Turner, Jasper Co. (H. S. Conard).
574. *P. Hydropiper* L. (*Persicaria* Opiz) Smart-weed or Water Pepper. An annual species; not very common.
575. *P. âcre* HBK. (*P. punctatum* Ell., *Persicaria punctata* Small) Our commonest Smart-weed; usually perennial.
576. *P. hydropiperoides* Michx. (*Persicaria* Small) Mild Water Pepper. Infrequent.
- *577. *P. Persicaria* L. (*Persicaria Persicaria* Small) Lady's Thumb. Common in cultivated ground and waste places. (Europe).
- *578. *P. orientale* L. (*Persicaria* Spach) Prince's Feather. A common escape from cultivation. (S. Asia).
579. *P. virginianum* L. (*Tovara* Raf.) Common in woods.
580. *P. sagittatum* L. (*Tracaulon* Small) Common in swampy places near timber.
- *581. *P. Convolvulus* L. (*Tiniaria* Webb. & Moquin.; *Bilderdykia* Dum.) Black Bindweed. Common; fields and waste places. (Europe)
582. *P. scandens* L. (*Tiniaria* Small; *Bilderdykia* Greene) High-climbing Wild Buckwheat. Common in thickets.
- *583. *P. dumetorum* L. (*Tiniaria* Opiz; *Bilderdykia* Dum.) Rare; resembling the preceding but with smaller flowers and fruit. (Europe).

Fagopyrum (Tourn.) L. Buckwheat.

- *584. *F. esculentum* Moench. Common Buckwheat. Persists as a weed for sometime after cultivation. (Probably N. Asia).

Polygonella Michx.

585. *P. articulata* (L.) Meisn. (*Delopyrum* Small) Very rare; Steamboat Rock, Hardin Co. (Miss C. M. King); Clinton Co. (Fred Weiss)

76. CHENOPODIACEAE (*Goosefoot Family*)**Cycloloma** Moq.

- *586. *C. atriplicifolium* (Spreng.) Coult. Winged Pigweed. Infrequent southeast.

Kochia Roth.

- *587. *K. Scoparia* L. The typical form rare; R.R. yards, Nevada, Story Co. (R. I. Cratty, Miss Ada Hayden) Introduced from Europe.
 *588. *K. trichophylla* Staph. (*K. scoparia* var. Bailey) The Burning Bush, a common escape from gardens. (Europe).

Chenopodium (Tourn.) L. Goosefoot, Pigweed.

- *589. *C. ambrosioides* L. Mexican Tea. Frequent. (Tropical Am.)
 *590. *C. ambrosioides anthelminticum* (L.) Gray. Wormseed. The more common form. (Tropical Am.)
 *591. *C. Botrys* L. Jerusalem Oak. Feather Geranium. Infrequent. (Europe).
 592. *C. rubrum* L. Rare; Emmet Co. (B. O. Wolden).
 *595. *C. glaucum* L. Oak-leaved Goosefoot. Rare. (Europe).
 594. *C. hybridum* L. Common, especially in rich woods.
 *595. *C. album* L. (including *C. lanceolatum* Muhl. and *C. paganum* Reich) Lamb's Quarters. Very common in rich soil, waste places, and around dwellings; a variable species. (Europe).
 *596. *C. urbicum* Moq. Infrequent. (Europe).
 597. *C. Boscianum* Moq. Common in woods.
 598. *C. leptophyllum* Nutt. Infrequent in or near timber. Leaves narrow.

Atriplex (Tourn.) L. Orach.

- *599. *A. rosea* L. Infrequent in waste places. Emmet, Cass, Polk and Sioux counties. (Europe).
 *600. *A. patula* L. Frequent as a weed. (Europe).
 *601. *A. patula hastata* (L.) Gray. A frequent weed. (Europe).

Axyris L.

- *602. *A. amaranthoides* L. Russian Pigweed. Introduced from northern Asia. Rare; Crawford Co. (L. H. Pammel).

Salsola L.

- *603. *S. pestifer* Aven Nelson (*S. Kali tenuifolia* of Manuals). Russian Thistle. Common in very dry situations, railway embankments, etc. Very troublesome in the Dakotas and westward. (Europe).

77. AMARANTHACEAE (*Amaranth Family*)**Amaranthus** (Tourn.) L. Amaranth.

- *604. *A. retroflexus* L. Pigweed, Red-root. Very common in rich soil and waste places. (Tropical Am.)
 *605. *A. hybridus* L. (including *A. paniculatus* L. and *A. hypochondriacus* L.) Frequent in cultivated ground. (Tropical Am.).
 606. *A. graecizans* L. (*A. albus* L.) Our common Tumble-weed; very widely distributed.
 *607. *A. blitoides* S. Wats. Prostrate Pigweed. Common. Native west of the Rocky Mountains.
 *608. *A. spinosus* L. Infrequent. Naturalized from Tropical America.

Acnida L.

609. *A. altissima* Riddell. (*A. tuberculata* Moq.) Water Hemp. Frequent in low ground.
 610. *A. tamariscina* (Nutt.) Wood. Frequent in very wet soil. The fruit dehiscing circumscissile, otherwise much like the preceding. Both species are dioecious.

Froelichia Moench.

611. *F. campestris* Small. (*F. floridana* Moq. in part). Rare in the southeastern counties.
 612. *F. gracilis* Moq. Rare in the southeast.

78. NYCTAGINACEAE (*Four-O'Clock Family*)**Allionia Loeffl.** (*Oxybaphus* L'Her.)

613. *A. linearis* Pursh. (*Oxybaphus* Robs.) Rare on dry hills; Woodbury Co. (L. H. Pammel).
 614. *A. hirsuta* Pursh. (*Oxybaphus* Sweet) Frequent in dry ground.
 615. *A. nyctaginea* Michx. (*Oxybaphus* Sweet) Wild Four-O'Clock. Frequent. The plant has a remarkably large root.

81. PHYTOLACCACEAE (*Pokeweed Family*)**Phytolacca L.**

616. *P. decandra* L. Pokeweed. Frequent in the southern part. Also cultivated. The juice of the ripe berries furnishes a bright red stain; the root very poisonous.

82. AIZOACEAE (*Carpet-weed Family*)**Mollugo L.**

- *617. *M. verticillata* L. Carpet-weed. Frequent on sandy shores. Native farther south. (Southern U. S.).

83. PORTULACACEAE (*Purslane Family*)**Talinum Adans.**

618. *T. parviflorum* Nutt. Very rare in dry ground; Lyon Co. (L. H. Pammel, R. I. Cratty.)

Claytonia (Gronov.) L.

619. *C. robusta* (Somes) Rydb. Rare; Johnson Co. Perhaps only a robust form of the following.
 620. *C. virginica* L. Spring Beauty. Common in low, rich woods; one of our earliest spring flowers.

Portulaca (Tourn.) L.

- *621. *P. oleracea* L. Purslane, Pigweed. Very common as a weed in gardens. (Europe).

85. CARYOPHYLLACEAE (*Pink Family*)**Anychia Michx.**

622. *A. canadensis* (L.) BSP. (*A. dichotoma* Michx.) Forked Chickweed. Frequent.

Stellaria

- *623. *S. media* (L.) Cyrill. Common and very troublesome in lawns. (Europe).
 624. *S. longifolia* Muhl. Frequent in low, grassy places.
 *625. *S. graminea* L. Rare; introduced in lawn grass seed. Emmet Co. (B. O. Wolden) (Europe).

Cerastium L. Mouse-ear Chickweed.

626. *C. nutans*. Raf. Frequent.
 *627. *C. vulgatum* L. A frequent weed in lawns and pastures. (Europe).
 628. *C. velutinum* Raf. (*C. oblongifolium* Torr.) Rare; Winneshiek Co. (E. W. Holway).

Arenaria L. Sandwort.

629. *A. lateriflora* L. (*Moehringia* Fenzl.) Frequent in the eastern part.
 *630. *A. serphyllifolia* L. Rare on a dry ridge in a wooded pasture; Emmet Co. (B. O. Wolden) Adventive from Europe.
 631. *A. stricta* Michx. (*Sabulina* Small) Infrequent in dry soil; Winneshiek and Fayette counties.

Agrostemma L.

- *632. *A. Githago* L. Corn Cockle. A frequent weed in grain fields. (Europe).

Silene L.

633. *S. antirrhina* L. Sleepy Catchfly; Common.
 634. *S. antirrhina divaricata* Robinson. Rare; Wapello Co. (L. H. Pammel).
 *635. *S. dichotoma* Ehrh. Frequent as a weed. (Europe).
 *636. *S. noctiflora* L. Night-flowering Catchfly. Infrequent. (Europe).
 637. *S. stellata* (L.) Ait. fil. A common woodland species. The leaves whorled.
 638. *S. nivea* (Nutt.) Otth. (*S. alba* Muhl.) Infrequent. Perhaps not native with us.
 *639. *S. Cserei* Baumg. Rare; Emmet and Chickasaw counties. (Europe).

Lychnis (Tourn.) L.

- *640. *L. alba* Mill. (*L. vespertina* Sibth. (*Melandrium* Gareke), White Campion. Frequent in hedges and around dwellings. (Europe).

Saponaria L.

- *641. *S. officinalis* L. Bouncing Bet. Common in cultivation and as an escape. (Europe).
 *642. *S. Vaccaria* L. (*Vaccaria vulgaris* Host., *V. Vaccaria* Britton.) Cow Herb. Frequent as a weed in grain fields. (Europe).

86. NYMPHAEACEAE (*Pond Lily Family*)**Nymphaea** (Tourn.) L.

643. *N. advena* Ait. (*Nuphar* Ait. fil.) Yellow Water Lily, Cow Lily. Common in shallow lakes and ponds.

Castalia Salisb.

644. *C. odorata* (Ait.) Woodville & Wood. (*Nymphaea* Ait.) Sweet-scented Water Lily. Rare in permanent ponds; Winneshiek Co. (H. Goddard).
 645. *C. tuberosa* (Paine) Greene. (*Nymphaea* Paine) Large White Water Lily. Common in lakes and ponds.

Nelumbo (Tourn.) Adans.

646. *N. lutea* (Willd.) Pers. Yellow Nelumbo, Water Chinquapin. Rare; found only in a few localities. Perhaps introduced into Iowa by the Indians who used its seeds and tubers for food.

Brasenia Schreb.

647. *B. Schreberi* Gmel. (*B. peltata* Pursh) Water Shield. Rare; Little Wall Lake, Hamilton Co. It is also native in Asia, Africa and Australia.

87. CERATOPHYLLACEAE (*Honewort Family*)**Ceratophyllum** L.

648. *C. demersum* L. Honewort. Frequent in shallow water.

89. RANUNCULACEAE (*Crowfoot Family*)**Ranunculus** (Tourn.) L.

649. *R. circinatus* Sibth. (*Batrachium* Rehb.) Stiff Water-Crowfoot. Infrequent.
650. *R. aquatilis trichophyllus* Gray. (*Batrachium trichophyllum* Bosch.) White Water-Crowfoot. Frequent in ponds.
651. *R. Cymbalaria* Pursh. (*Oxygraphis* Prantl; *Halerpestes* Greene). Frequent on low prairies.
652. *R. delphinifolius* Torr. (*R. multifidus* Pursh) Yellow Water-Crowfoot. Frequent in still water, sloughs and ponds.
653. *R. Purshii* Richards. Rare; known in Iowa only near the s.e. shore of Spirit Lake, Dickinson Co. (R. I. Cratty).
654. *R. ovalis* Raf. (*R. rhomboideus* Goldie) Frequent on dry hill-sides.
655. *R. sceleratus* L. Cursed Crowfoot. Common in woods.
656. *R. abortivus* L. Common in low, moist woods.
657. *R. recurvatus* Poir. Infrequent.
658. *R. fascicularis* Muhl. Frequent on hill slopes.
659. *R. septentrionalis* Poir. Very common in low ground. Our earliest Yellow Buttercup.
660. *R. caricetorum* Greene. Rare; Winneshiek Co. (H. Goddard) Closely allied to the preceding, the stems and petioles pubescent. Referred here by the late Dr. Rydberg.
661. *R. sicaeformis* Mack. & Bush. Rare; Fayette Co. (Bruce Fink) Perhaps only a rank form of *R. septentrionalis*.
662. *R. pennsylvanicus* L. fil. Frequent.
- *663. *R. acris* L. Infrequent. (Europe).

Myosurus (Dill.) L.

664. *M. minimus* L. Mouse-tail. Very rare; Muscatine Co. (Ferd Reppert).

Thalictrum (Tourn.) L. Meadow-Rue.

665. *T. dioicum* L. Common in rich woods.
666. *T. dasycarpum* Fisch. & Lall. Very common, woods and prairies.
667. *T. hypoglaucom* Rydb. Rare; Story and Grundy counties. (det. by Dr. Rydberg).

Anemonella Spach.

668. *A. thalictroides* (L.) Spach. (*Syndesmon* Hoffmg. Rue Anemone. Common in low woods. An early spring flower.

Hepatica (Rupp.) Hill.

669. *H. acutiloba* DC. Hepatica. Common in low, rich woods.

Anemone (Tourn.) L. Anemone.

670. *A. caroliniana* Walt. Infrequent in or near timber.

- 671. *A. cylindrica* Gray. Common; woods and prairies.
- 672. *A. virginiana* L. Frequent; resembling the preceding, but much coarser.
- 673. *A. canadensis* L. Very common; open woods and prairies.
- 674. *A. quinquefolia* L. Common in low, rich woods.

Pulsatilla Pers.

- 675. *P. patens* (L.) Mill. (*Anemone patens* *Wolfgangiana* of Gray's Manual) Wind or Pasque Flower. Common in early spring on dry hillsides, especially northward.

Clematis L. Virgin's Bower.

- 676. *C. virginiana* L. (including *C. missouriensis* Rydb.) Common in open woods.
- 677. *C. Pitcheri* T. & G. (*Viorna* Britton) Frequent.
- 678. *C. verticillaris* DC. Infrequent.

Isopyrum L.

- 679. *I. biternatum* (Raf.) T. & G. Common in rich woods.

Caltha (Rupp.) L.

- 680. *C. palustris* L. Marsh Marigold. Frequent in springy places.

Aquilegia (Tourn.) L.

- 681. *A. canadensis* L. (*A. latiuscula* Greene) Columbine. Very common in open woods.

Delphinium (Tourn.) L. Larkspur.

- *682. *D. Ajacis* L. An occasional escape from gardens. (S. Europe).
- 683. *D. tricornis* Michx. Infrequent in the eastern part.
- *684. *D. cultorum* Voss. The common garden cultivar; occasionally escaped; origin not worked out.
- 685. *D. virescens* Nutt. (*D. albescens* Rydb.; has been referred to the western *D. Penardi* Huth.) Frequent in dry soil; open woods and prairies.

Aconitum (Tourn.) L.

- 686. *A. noveboracense* Gray (var. *pseudociliatum* Fassett). Aconite. Monkshood. Infrequent; Dubuque and Allamakee counties.

Actaea L. Baneberry.

- 687. *A. rubra* (Ait.) Willd. The common red-fruited Baneberry.
- 688. *A. alba* (L.) Mill. (*A. rubra neglecta* Robinson) Rare in rich woods; resembles the preceding, but with white fruit.
- 689. *A. brachypoda* Ell. (*A. alba* Bigel. not Mill.) Frequent. The white fruit on thickened pedicels.

Hydrastis Ellis.

- 690. *H. canadensis* L. Golden Seal. Rare in the northeast.

91. BERBERIDACEAE (*Barberry Family*)**Podophyllum** L.

- 691. *P. peltatum* L. May Apple, Mandrake. Common in open woods, except in the northwestern part.

Jeffersonia B.S. Barton.

- 692. *J. diphylla* (L.) Pers. Twin-leaf. Rare; Clermont, Fayette Co. (E. R. Walker).

Caulophyllum Michx.

- 693. *C. thalictroides* (L.) Michx. Blue Cohosh. Frequent in rich woods.

Berberis (Tourn.) L.

- *694. *B. vulgaris* L. Common Barberry. A frequent escape from cultivation. It is the winter host of the stem rust of wheat. (Europe).
- *695. *B. Thunbergii* DC. Japanese Barberry. Very common in cultivation and occasionally escaped. (Japan).

92. MENISPERMACEAE (*Moonseed Family*)**Menispermum** (Tourn.) L.

- 696. *M. canadense* L. Moonseed. A perennial-stemmed vine, common in open woods.

96. ANONACEAE (*Custard Apple Family*)**Asimina** Adans.

- 697. *A. triloba* Dunal. Papaw. Infrequent in the southern part.

102. PAPAVERACEAE (*Poppy Family*)**Sanguinaria** (Dill.) L.

- 698. *S. canadensis* L. Bloodroot. Very common in rich woods.

Chelidonium (Tourn.) L.

- *699. *C. majus* L. Celandine; rare as an escape. (Europe).

Argemone L.

- *700. *A. mexicana* L. Mexican Poppy. Rare as a garden escape. (Mexico).
- 701. *A. intermedia* Sweet. Rare on our western border.

102a. FUMARIACEAE (*Fumitory Family*)**Dicentra** Bernh.

- 702. *D. Cucullaria* (L.) Bernh. (*Bicuculla* Millsp.) Dutchman's Breeches. A very common spring flower in low, rich woods.
- 703. *D. canadensis* (Goldie) Walpers. (*Bicuculla* Millsp.) Squirrel corn. Infrequent in the northeast.

Corydalis (Dill.) Medic.

- 704. *C. micrantha* (Engelm.) Gray. (*Capnoides* Britton). Frequent in dry ground.
- 705. *C. aurea* Willd. (*Capnoides* Kuntze) Infrequent.

103. CRUCIFERACEAE (*Mustard Family*)**Draba** (Dill.) L.

- *706. *D. verna* L. Whitlow Grass. Rare. (Europe).
- 707. *D. caroliniana* Walt. Infrequent.

Berteroa DC.

- *708. *B. incana* (L.) DC. Frequent in cultivated ground. (Europe).

Alyssum (Tourn.) L.

- *709. *A. alyssoides* L. (*A. calycinum* L.) Sweet Alyssum. A rare escape as a weed in pastures, Monona Co. (Europe).

Lesquerella S. Wats.

- 710. *L. argentea* (Pursh) MacM. (*L. ludoviciana* S. Wats.) Very rare; Iowa Falls, Hardin Co. (Morton E. Peck).

Thlaspi (Tourn.) L.

- *711. *T. arvense* L. Penny Cress. Frequent. (Europe).

Lepidium (Tourn.) L. Pepper grass.

- 712. *L. virginicum* L. Common.

713. *L. densiflorum* Schrad. (*L. apetalum* of authors, not Willd.) Very common.
- *714. *L. perfoliatum* L. Rare; Story and Poweshiek counties. (Europe).
- *715. *L. campestre* R. Br. Infrequent. (Europe).
- *716. *L. Draba* L. (*Cardaria* Desv.) A noxious perennial species becoming common. (Europe).
- Capsella** Medic.
- *717. *C. Bursa-pastoris* (L.) Medic. (*Bursa* Britton) Shepherd's Purse. A very common weed. (Europe).
- Camelina** Crantz.
- *718. *C. sativa* (L.) Crantz. Common, especially in flax fields. (Europe).
- *719. *C. microcarpa* Andr. Rare; Dickinson Co. (H. S. Conard). (Europe).
- Raphanus** (Tourn.) L.
- *720. *R. Raphanistrum* L. Wild Radish, Jointed Charlock. Rare. (Europe).
- *721. *R. sativus* L. The Common Radish; occasionally persists around gardens. (Europe).
- Chorispora** R. Br.
- *722. *C. tenella* DC. Rare; on Federal Highway No. 75, two mi. n. of Sioux City. (B. O. Wolden) Adventive from Europe.
- Brassica** (Tourn.) L. Mustard.
- *723. *B. alba* (L.) Boiss. White Mustard. Rare as an escape. (Europe).
- *724. *B. arvensis* (L.) Ktze, Charlock. A noxious weed, very common in grain fields and waste places. (Europe).
- *725. *B. juncea* (L.) Cosson. Indian Mustard. Frequent; often confused with the preceding. (Europe).
- *726. *B. nigra* (L.) Koch. Black Mustard. Roadsides and waste places. Less common than *B. arvensis*. (Europe).
- *727. *B. campestris* L. Rutabaga. Occasionally persisting one or two years. (Europe).
- *728. *B. Napus* L. Rape. Common in cultivation and as an escape. (Europe).
- *729. *B. Rapa* L. Turnip. Occasionally found like the two preceding. (Europe).
- Eruca** (Tourn.) Mill.
- *730. *E. sativa* Mill. Garden Rocket. Sparingly adventive. (Europe).
- Diplotaxis** DC.
- *731. *D. muralis* (L.) DC. Infrequent in waste places (Europe).
- Conringia** (Heist.) Link.
- *732. *C. orientalis* (L.) Dumort. Hare's-ear Mustard. Infrequent. (Europe).
- Sisymbrium** (Tourn.) L.
- *733. *S. officinale leiocarpum* DC. Hedge Mustard. Common; all ours have smooth pods. (Europe).
- *734. *S. altissimum* L. Tumble or Tower Mustard. Common in waste places. (Europe).
735. *S. canescens* Nutt. (*Sophia pinnata* Howell) A common polymorphous species, in rather dry ground. Seeds in two rows.
- *736. *S. Sophia* L. (*Sophia Sophia* Britton) Infrequent. (Europe).

- *737. *S. Thalianum* (L.) J. Gay. (*Arabidopsis* Schur.) Rare; Webster Co. (F. W. Paige) (Europe).
 738. *S. incisum* Engelm. Infrequent in dry ground. Seeds in a single row in pod.

Hesperis (Tourn.) L.

- *739. *H. matronalis* L. Dame's Rocket. An occasional escape. (Europe).

Erysimum (Tourn.) L. (*Cheirinia* Link)

740. *E. cheiranthoides* L. Worm-seed Mustard. Common in dry, sandy soil.
 *741. *E. parviflorum* Nutt. Rare; introduced along railroads. (Western Plains).
 742. *E. asperum* DC. Western Wall Flower. Rare as a native on a rocky ledge, Van Buren Co. (Mrs. A. A. Campbell). Occasionally cultivated.

Radicula (Dill.) Hill. (*Nasturtium* R. Br.)

- *743. *R. nasturtium-aquaticum* (L.) Britten and Rendle. Infrequent in the northeast. (Europe).
 *744. *R. amphibia* (L.) Druce. (*Nasturtium* L.; *Rorippa* Bess.) Muddy banks of the Des Moines R., Estherville, Emmet Co. (B. O. Wolden) A recent immigrant. (Europe).
 *745. *R. sylvestris* (L.) Druce. Yellow Cress. Rare in waste places. (Europe).
 746. *R. sessiliflora* (Nutt.) Greene. Rare; Decatur Co. (J. P. Anderson).
 747. *R. sinuata* (Nutt.) Greene. Infrequent.
 748. *R. palustris* (L.) Moench. Marsh Cress. Common in low fields.
 *749. *R. Armoracia* (L.) Robinson. (*Armoracia rusticana* Gaertn.) Horse Radish. Frequently escaped. (Europe).

Barbarea R. Br.

- *750. *B. vulgaris* R. Br. Winter Cress. Introduced in Iowa, but native farther north. (Europe).

Iodanthus T. & G.

751. *I. pinnatifidus* (Michx.) Steud. (*Thelypodium* S. Wats.) Infrequent.

Dentaria (Tourn.) L.

752. *D. laciniata* Muhl. Toothwort. Frequent in low, wet ground.

Cardamine (Tourn.) L. Bitter Cress.

753. *C. bulbosa* (Schreb.) BSP. (*C. rhomboidea* Goldie) Common in low, wet places.
 754. *C. Douglassii* (Torr.) Britton. (*C. purpurea* Britton) Purple Cress. Quite rare; Linn Co. (Ruth E. Whaley).
 755. *C. parviflora* L. (*C. hirsuta sylvatica* of authors). Infrequent in woods.
 756. *C. pennsylvanica* Muhl. Frequent in open woods.

Arabis L. Rock Cress.

757. *A. lyrata* L. Rare in the northeast.
 758. *A. dentata* T. & G. Frequent in woods.
 759. *A. Drummondii* Gray. Infrequent in open woods.
 760. *A. brachycarpa* (T. & G.) Britton. Infrequent. Basal leaves densely pubescent.
 761. *A. hirsuta* (L.) Scop. Frequent in rather dry ground.

762. *A. glabra* (L.) Bernh. (*Turritis* L.) Rare; Allamakee Co. (H. A. Hendricksen).
 763. *A. laevigata* (Muhl.) Poir. Infrequent.
 764. *A. canadensis* L. Sickle-pod. Infrequent. The Ledges, Boone Co. (L. H. Pammel).

105. CAPPARIDACEAE (*Caper Family*)

Polanisia Raf.

765. *P. graveolens* Raf. Infrequent; Lake shores and sandy banks.
 766. *P. trachysperma* T. & G. Similar situations and much more common.

Cleome L.

767. *C. serrulata* Pursh. (*Peritoma* DC.) Stinking Clover, Spider Plant. Native in the western part, also cultivated.

106. RESEDACEAE (*Mignonette Family*)

Reseda (Tourn.) L.

- *768. *R. Luteola* L. Dyer's Weed. Rare as an introduced weed; Monona Co. (R. E. Pim) (Europe).

113. CRASSULACEAE (*Orpine Family*)

Penthorum (Gronov.) L.

769. *P. sedoides* L. Ditch Stone-crop. Common in low places.

Sedum (Tourn.) L.

- *770. *S. purpureum* Tausch. (*S. Telephium* of Gray's Manual 6th not L.) Live-for-ever. Rare as an escape from cultivation. (Europe).

115. SAXIFRAGACEAE (*Saxifrage Family*)

Sullivantia T. & G.

771. *S. renifolia* Rosendahl. (*S. ohionis* T. & G. in part). Rare in the northeastern part.

Saxifraga (Tourn.) L.

772. *S. pennsylvanica* L. (*Micranthes* Haw.) Saxifrage. Infrequent; eastern and southern.

Heuchera L.

773. *H. hispida* Pursh. Alum Root. Common in dry ground.

Mitella (Tourn.) L.

774. *M. diphylla* L. Miterwort. Common in the eastern half.

Chrysosplenium (Tourn.) L.

775. *C. tetrandrum* Fries. (*C. iowense* Rydb.) Golden Saxifrage. Rare; Winneshiek Co. (Holway, Goddard).

Parnassia (Tourn.) L.

776. *P. caroliniana* Michx. (*P. americana* Muhl.) Grass of Parnassus. Once common in low ground, but rapidly disappearing.

Ribes L.

777. *R. Cynosbati* L. (*Grossularia* Mill.) Prickly-fruited Gooseberry. Frequent in open woods.
 778. *R. missouriense* Nutt. (*Grossularia* Coville & Britton; *Ribes gracile* Michx.) Our very common Smooth-fruited Gooseberry.
 779. *R. oxyacanthoides* L. (*Grossularia* Mill.) Smooth Gooseberry. Rare in Balsam Fir Grove, Allamakee Co. (L. H. Pammel).
 780. *R. americanum* Mill. (*R. floridum* L'Her.) Wild Black Currant. Common.

- *781. *R. vulgare* Lam. The Red Currant of gardens. An occasional escape. (Europe).
- *782. *R. odoratum* Wend. (*R. aureum* of authors not Pursh). Flowering Currant. A frequent escape. (Western U. S.).

121. HAMAMELIDACEAE (*Witch-Hazel Family*)**Hamamelis** L.

- 783. *H. virginiana* L. Witch-hazel. Infrequent in the eastern part.

122. PLATANACEAE (*Plane-tree Family*)**Platanus** (Tourn.) L.

- 784. *P. occidentalis* L. Sycamore. Common in the southern half.

124. ROSACEAE (*Rose Family*)**Physocarpus** Maxim.

- 785. *P. opulifolius intermedius* (Rydb.) Robinson. (*Opulaster intermedius* Rydb.) Ninebark. Common, especially in the eastern part.

Spiraea (Tourn.) L.

- 786. *S. alba* Du Roi. (*S. salicifolia* of Am. authors not of L. which is an Asiatic species). Common.

Aruncus (L.) Adans.

- 787. *A. sylvester* Kost. (*A. pubescens* Rydb.) Goat's Beard. Rare in wooded ravines in the eastern part.

Pyrus (Tourn.) L.

- *788. *P. baccata* L. Siberian Crab Apple. Rare as an escape. (Asia).
- 789. *P. ioensis* (Wood) Bailey. (*Malus* Britton) Wild Crab Apple. Frequent except in the northwestern part.
- *790. *P. Malus* L. (*Malus sylvestris* Mill.) The Common Apple. An occasional escape. (Eurasia).

Sorbus (Tourn.) L.

- 791. *S. americana* L. (*Pyrus* DC.) American Mountain Ash. Rare; Allamakee and Fayette counties.
- *792. *S. Aucuparia* L. (*Pyrus* Ehrh.) European Mountain Ash or Rowan Tree. Rarely escaped. (Europe).

Amelanchier Medic. June or Service Berry.

- 793. *A. canadensis* (L.) Medic. Common in woods, the early leaves pubescent.
- 794. *A. laevis* Wiegand. Frequent; a segregate from the preceding—leaves smooth from the first.
- 795. *A. oblongifolia* (T. & G.) Roem. Frequent in the northeastern part.
- 796. *A. humilis* Wiegand. (*A. alnifolia* of Gray's Manual, 7th ed.) Common except in the southwestern part.

Crataegus L. Thorn Apple. Hawthorn.

- 797. *C. Crus-galli* L. Cock-spur Thorn. Infrequent.
- 798. *C. punctata* Jacq. Common; one of our handsomest species.
- 799. *C. Margaretta* Ashe. Frequent.
- 800. *C. succulenta* Schrad. (*C. macracantha* of Manuals). Frequent.
- 801. *C. Calpodendron* (Ehrh.) Medic. (*C. tomentosa* of Am. authors not of L.) Frequent in the eastern part.
- 802. *C. pertomentosa* Ashe. Infrequent.

803. *C. chrysocarpa* Ashe. (*C. rotundifolia* Borek.; *C. coccinea rotundifolia* Sargent). Infrequent. Emmet Co. (R. I. Cratty, B. O. Wolden).

804. *C. mollis* (T. & G.) Scheele. Our commonest species. Much field work is needed in this difficult genus.

Fragaria (Tourn.) L. Strawberry.

805. *F. virginiana* Duchesne. Less common than the next.

806. *F. Grayana* Vilmorin (*F. virginiana illinoensis* Prince). Our commonest Wild Strawberry. This and the preceding variable.

807. *F. americana* (Porter) Britton. (*F. vesca americana* Porter). Common in woods.

Potentilla L. Cinquefoil, Five-finger.

808. *P. arguta* Pursh. Frequent in dry soil.

809. *P. monspeliensis* L. A very common, weedy species.

810. *P. millegrana* Engelm. (*P. rivalis* var. S. Wats.) Frequent in marshes.

811. *P. pentandra* (Engelm.) (*P. rivalis* var. S. Wats.) S. Wats. Frequent in low places.

812. *P. paradoxa* Nutt. Frequent on sandy shores of lakes.

813. *P. argentea* L. Rare in dry, barren soil.

- *814. *P. recta* L. Rare. Introduced in a pasture in Story Co. (L. H. Pammel). Adventive from Europe.

815. *P. palustris* (L.) Scop. (*Comarum* L.) Frequent in our northern marshes.

816. *P. tridentata* Ait. (*Sibbaldiopsis* Rydb.) Rare; near Hesper, Winneshiek Co. (A. S. Hitchcock).

817. *P. Anserina* L. (*Argentina* Rydb.) Silver Weed. Rare in moist situations.

818. *P. canadensis* L. (Including the var. *simplex* T. & G.). Very common; open woods and prairies.

Dasiphora Raf.

819. *D. fruticosa* (L.) Rydb. (*Potentilla* L.) Shrubby Cinquefoil. Rare in the northeastern section.

Geum L. Avens.

820. *G. canadense* Jacq. (*G. album* J. F. Gmelin). Common in woods.

821. *G. virginianum* L. Frequent.

822. *G. macrophyllum* Willd. Infrequent; Winneshiek and Jefferson counties.

823. *G. strictum* Ait. Frequent. The flowers of this and the preceding are yellow.

824. *G. camporum* Rydb. Rare; Emmet Co. (Cratty and Pammel). Rydberg in N. Am. Flora. XXII, 403 (1913).

Sieversia Willd.

825. *S. ciliata* (Pursh) G. Don. (Including *S. triflorum* R. Br., *Geum* Pursh) Purple Avens, Old Man's Whiskers. Infrequent; central and eastern.

Rubus (Tourn.) L.

826. *R. strigosus* Michx. (*R. idaeus aculeatissimus* Regel & Tiling) Wild Red Raspberry; very common in open woods.

827. *R. occidentalis* L. Wild Black Raspberry. Common with the preceding.

- 828. *R. triflorus* Richards. (*R. pubescens* Raf.) Rare; Dubuque Co. (Pammel & Trenk).
- 829. *R. allegheniensis* Porter. Our commonest Wild Blackberry.
- 830. *R. pergratus* Blanchard. Frequent.
- 831. *R. argutus* Link. (*R. villosus* of Authors). Infrequent.
- 832. *R. heterophyllus* Willd. (*R. recurvans* Blanchard). Frequent in the central part.
- 833. *R. Baileyanus* Britton. Infrequent. Referred here by M. L. Fernald.
- 834. *R. flagellaris* Willd. Frequent. The Iowa *Rubi* need a thorough revision, requiring careful field work.

Agrimonia (Tourn.) L. Agrimony.

- 835. *A. gryposepala* Wallr. Our commonest woodland species.
- 836. *A. striata* Michx. Infrequent in the southern part.
- 837. *A. parviflora* Ait. Frequent in the southern part.

Rosa (Tourn.) L. Rose.

- 838. *R. blanda* Ait. Smooth or Meadow Rose; Common, especially in or near timber.
- 839. *R. suffulta* Greene. (*R. pratincola* and *R. heliophila* Greene). Our common very prickly-stemmed species on upland prairie and in cultivated fields.
- 840. *R. Woodsii* Lindl. Rare.
- 841. *R. carolina* L. Swamp Wild Rose. Frequent southeast.
- 842. *R. Lyonii* Bush. Several specimens referred here by Mr. B. F. Bush.
- 843. *R. conjuncta* Rydb. Infrequent; central and western.
- 844. *R. virginiana* Mill. (*R. lucida* Ehrh.) Infrequent.
- 845. *R. Williamsii* Fernald. Two specimens referred here by Mr. Bush.
- *846. *R. rubiginosa* L. Sweet Brier, the Eglantine of Chaucer, Spenser, and Shakespere. An occasional escape. (Europe). The above is only a tentative list. Our Wild Roses require more thorough field study, and better specimens in both flower and fruit.

Prunus (Tourn.) L.

- 847. *P. virginiana* L. (*P. serotina* Ehrh.) Wild Black Cherry. Very common.
- 848. *P. nana* Du Roi. (*P. virginiana* of Authors, not of L.) Choke Cherry. Common, border of woods; the fruit very astringent.
- 849. *P. pennsylvanica* L. fil. Frequent in woods.
- 850. *P. pumila* L. Sand Cherry. Rare; Lyon and Dickinson counties.
- *851. *P. Cerasus* L. The Common Cultivated Cherry. An occasional escape. (Europe).
- 852. *P. americana* Marsh. Our common Wild Plum; border of woods.
- *853. *P. Persica* (L.) Stokes. (*Amygdalus* L.) Peach. Escaped in the southern part. (Asia).

126. LEGUMINACEAE (*Pea Family*)

Desmanthus Willd.

- 854. *D. illinoensis* (Michx.) (MacM.) (*Acuan* Kuntze). Infrequent; shores of lakes and ponds.

Schrankia Willd.

855. *S. uncinata* Willd. (*Leptoglottis Nuttallii* DC.) Sensitive Brier. Rare; Harrison Co. (Mrs. A. J. Schim).

Gymnocladus Lam.

856. *G. dioica* (L.) Koch. (*G. canadensis* L.). Kentucky Coffee Tree. Throughout, but not very common.

Gleditsia L.

857. *G. triacanthos* L. Honey Locust. Frequent.

Cassia (Tourn.) L.

858. *C. Medsgeri* Shafer. (*C. marilandica* of authors in part). Wild Senna. Infrequent in dry ground.
859. *C. Chamaecrista* L. (*Chamaecrista fasciculata* Greene) Part-ridge Pea. Common in dry ground.

Cercis L.

860. *C. canadensis* L. Red Bud or Judas Tree. Frequent in the southern part, and common in cultivation.

Baptisia Vent. False Indigo.

861. *B. bracteata* (Muhl.) Ell. (*B. leucophoea* Nutt.) Very common; open prairies and border of woods.
862. *B. leucantha* T. & G. Frequent in open woods.

Crotalaria (Dill.) L.

863. *C. sagittalis* L. Rattle-box. Infrequent in dry soil.

Lupinus (Tourn.) L.

864. *L. perennis* L. Lupine. Rare; Winneshiek Co. (E. W. Holway).

Trifolium (Tourn.) L.

- *865. *T. arvense* L. Rabbit-foot or Stone Clover. Frequent along highways. (Europe).
*866. *T. incarnatum* L. Crimson or Italian Clover. Infrequent as an escape. (Europe).
*867. *T. pratense* L. Red Clover. Very common in cultivation and as an escape. (Europe).
868. *T. reflexum* L. Buffalo Clover. Rare; Muscatine Co. (Ferd Reppert).
*869. *T. repens* L. Everywhere common. (Europe).
*870. *T. hybridum* L. Alsike Clover. Cultivated and escaped. (Europe).
*871. *T. agrarium* L. Yellow or Hop Clover. Infrequent in dry ground. (Europe).
*872. *T. procumbens* L. Low Hop Clover. Frequent along highways. The middle leaflet petioled. (Europe).

Melilotus (Tourn.) Hill.

- *873. *M. officinalis* (L.) Lam. Yellow Sweet Clover. Frequent in waste places and on roadsides. (Europe).
*874. *M. alba* Desr. White Sweet Clover. Common as a weed along highways, railroads and in waste places. (Europe). An annual form, Hubam Clover, is extensively cultivated for forage, and as a soiling crop.

Medicago (Tourn.) L.

- *875. *M. sativa* L. Alfalfa. Common in cultivation and as an escape. (Europe).
*876. *M. falcata* L. Rare; Story, Ringgold, and Hamilton counties. (Europe).

- *877. *M. lupulina* L. Black Medick. Frequent in lawns and as an escape. (Europe).

Anthyllis (Rivinius) L.

- *878. *A. Vulneraria* L. Kidney Vetch. Rare. Story and Wright counties. A beautiful plant. (Europe).

Hosackia Dougl.

879. *H. americana* (Nutt.) Piper. (*H. Purshiana* Benth.) Infrequent in dry ground.

Psoralea L.

880. *P. Onobrychis* Nutt. Rare in the southern part.
 881. *P. floribunda* Nutt. (*P. tenuifolia* var. Rydb.) Frequent in the western counties.
 882. *P. argophylla* Pursh. Common on upland prairies.
 883. *P. lanceolata* Pursh. Rare; Monona Co. (L. H. Pammel).
 884. *P. esculenta* Pursh. The Pomme Blanche, or Pomme de Prairie of the French Voyageurs. Frequent on knolls. The large, starchy root was used for food by the Indians.

Amorpha L.

885. *A. canescens* Pursh. Lead Plant. Very common on dry knolls and hillsides.
 886. *A. microphylla* Pursh. (*A. nana* Nutt.). Infrequent on prairies; Emmet and Decatur counties.
 887. *A. fruticosa* L. False Indigo. A very common shrub.
 888. *A. fruticosa angustifolia* Pursh. Rare; a narrow-leaved form of the preceding.

Dalea Juss.

889. *D. alopecuroides* Willd. (*Parosela Dalea* Britton). Frequent near timber.
 890. *D. enneandra* Nutt. (*D. laxiflora* Pursh, *Parosela enneandra* Britton). Infrequent in dry soil in the western part.

Petalostemon Michx. Prairie Clover.

891. *P. purpureus* (Vent.) Rydb. Very common, especially on prairies.
 892. *P. multiflorus* Nutt. Very rare; Woodbury Co. (L. H. Pammel.)
 893. *P. oligophyllus* (Torr.) Rydb. Rare; Woodbury and Monona counties.
 894. *P. candidus* Michx. White Prairie Clover. Common.

Tephrosia Pers.

895. *T. virginiana* (L.) Pers. (*Cracca* L.) Hoary Pea. Infrequent; Delaware, Lee and Buchanan counties.

Robinia L.

- *896. *R. Pseudo-Acacia* L. Common Locust or False Acacia. Cultivated and escaped. (Southern U. S.).
 *897. *R. hispida* L. Bristly Locust or Rose Acacia. Rare as an escape from cultivation. Chickasaw Co. (W. D. Spiker). (Southern U. S.).

Astragalus (Tourn.) L.

898. *A. caryocarpus* Ker. (*Geoprimum crassicaupus* Rydb.) Ground Plum. Frequent on dry prairies and morainic hills; northern and western sections.

- 899. *A. canadensis* L. (*A. carolinianus* L.) Milk Vetch. Common on upland soil.
- 900. *A. adsurgens* Pall. (*A. striatus* Nutt.) Rare; Ocheydan Mound, Osceola Co. (T. J. Fitzpatrick).
- 901. *A. Hypoglottis* L. Rare; open prairies, Emmet and Kossuth counties.
- 902. *A. distortus* T. & G. Infrequent in dry soil.
- 903. *A. lotiflorus* Hook. (*Phaca* Nutt.). Rare in the southwest section; Decatur and Fremont counties.

Oxytropis DC.

- 904. *O. Lamberti* Pursh. Loco or Crazy Weed. Frequent in the northwest section on morainic hills.

Glycyrrhiza (Tourn.) L.

- 905. *G. lepidota* (Nutt.) Pursh. Wild Licorice. Frequent in rather dry soil. The fruit resembling cocklebur.

Coronilla L.

- *906. *C. varia* L. Axseed or Axwort. A recent immigrant from Europe; Plymouth, Monona and Lyon counties. (Europe).

Desmodium Desv. (Meibomia Heist.)

- 907. *D. nudiflorum* (L.) DC. (*M. Kuntze*). Infrequent in woods.
- 908. *D. grandiflorum* (Walt.) DC. (*M. Kuntze*). Common in woods.
- 909. *D. canescens* (L.) DC. (*M. Kuntze*). Rare in the southern part.
- 910. *D. bracteosum longifolium* (T. & G.) Robinson. (*M. longifolia* Vail). Frequent in the eastern half.
- 911. *D. illinoense* Gray (*M. Kuntze*). Infrequent; flowers occasionally yellow.
- 912. *D. Dillenii* Darl. (*M. Kuntze*). Frequent in rich woods.
- 913. *D. paniculatum* (L.) DC. (*M. Kuntze*). Infrequent.
- 914. *D. canadense* (L.) DC. (*M. Kuntze*). Very common, prairies and open woods.

Lespedeza Michx. Bush Clover.

- 915. *L. violacea* (L.) Pers. Frequent in dry soil in the southern part.
- 916. *L. virginica* (L.) Britton. Infrequent. Mostly in the southern part.
- 917. *L. capitata* Michx. Common; dry prairies and margin of woods.
- 918. *L. capitata longifolia* (DC.) T. & G. Infrequent; a narrow-leaved form of the preceding.
- 919. *L. leptostachya* Engelm. Rare on upland prairies.

Vicia (Tourn.) L. Vetch.

- *920. *V. sativa* L. Spring Vetch. Infrequent as an escape from cultivation. (Europe).
- 921. *V. americana* Muhl. Very common.
- 922. *V. americana angustifolia* Nees. (*V. linearis* S. Wats., *V. sparsiflora* Nutt.) Rare; Marshall Co. (L. H. Pammel).
- *923. *V. villosa* Roth. Hairy or Winter Vetch. Cultivated, and a frequent escape. (Europe).

Lathyrus (Tourn.) L. Wild Vetch.

- 924. *L. palustris* L. Frequent on low prairies; becoming rare.
- 925. *L. ochroleucus* Hook. Frequent in woods.
- 926. *L. venosus* Muhl. Common; prairies and in open woods.

Apios (Boerh.) Ludwig.

927. *A. tuberosa* Moench. Ground Nut or Wild Bean. Frequent; border of woods, climbing over shrubs.

Strophostyles Ell. Trailing Bean.

928. *S. helvola* (L.) Britton. Frequent; sandy shores of lakes and streams.
 929. *S. pauciflora* (Benth.) S. Wats. Frequent; with the preceding.

Amphicarpa Ell. Hog Peanut.

930. *A. monoica* (L.) Ell. (*Falcata comosa* Kuntze). Common, prairies and open woods.
 931. *A. Pitcheri* T. & G. (*Falcata* Kuntze). Frequent. Often confused with the preceding.

127. GERANIACEAE (*Geranium Family*)**Geranium** (Tourn.) L. Cranesbill.

932. *G. maculatum* L. Wild Cranesbill. Very common in low, rich woods.
 933. *G. carolinianum* L. Frequent; mostly in dry soil.
 934. *G. Bicknellii* Britton. Infrequent; Emmet, Fayette, and Montgomery counties.
 *935. *G. pusillum* Burm. fil. Rare; introduced with lawn grass seed, Emmet and Story counties. (Europe).

Erodium L'Her.

- *936. *E. cicutarium* (L.) L'Her. Storksbill, Alfilaria, Filaree. Rare as an introduced plant. Cultivated in the Pacific Southwest. (Europe).

128. OXALIDACEAE (*Wood-Sorrel Family*)**Oxalis** L.

- *937. *O. corniculata* L. (*O. repens* of Gray's Manual 7th; *Xanthoxalis* Small) Creeping Wood Sorrel. Introduced around greenhouses. (Europe).
 938. *O. cymosa* Small. (*O. corniculata* of Gray's Manual in part, *Xanthoxylis* Small). Tall Yellow Wood Sorrel. Our commonest species and very variable.
 939. *O. stricta* L. (*O. Bushii* Small in part, *Xanthoxalis* Small) A low, variable plant, with closely appressed pubescence on stems and branches. Frequent in dry ground.
 940. *O. violacea* L. (*Ionoxalis* Small). Violet Wood Sorrel. Common, woods and open fields.

The Yellow Wood Sorrels form an extremely variable group; constant characters seem to be lacking, so that with our present knowledge it is only possible to list them provisionally.

130. LINACEAE (*Flax Family*)**Linum**

- *941. *L. usitatissimum* L. Common Flax. A frequent escape from cultivation. (Europe).
 942. *L. sulcatum* Riddell. (*Cathartolinum* Small) Frequent on dry prairies.
 943. *L. virginianum* L. (*Cathartolinum* Reichenb.) Infrequent; Polk and Des Moines counties.

133. ZYGOPHYLLACEAE (*Caltrop Family*)**Tribulus** (Tourn.) L.

- *944. *T. terrestris* L. Caltrop, Puneture Vine. Infrequent in dry ground. The spiny burs adhering to automobile and bicycle tires, hence the second popular name. (Europe).

135. BUTACEAE (*Rue Family*)**Zanthoxylum** L.

945. *Z. americanum* Mill. Prickly Ash. Very common in open woods.

Ptelea L.

946. *P. trifoliata* L. Hop Tree. Infrequent in the eastern and southern sections.

136. SIMARUBACEAE (*Quassia Family*)**Ailanthus** Desf.

- *947. *A. altissima* (Mill.) Swingle. (*A. glandulosa* Desf.). Tree of Heaven. Rare as an escape from cultivation; a native of China and the East Indies.

143. POLYGALACEAE (*Milkwort Family*)**Polygala** (Tourn.) L. Milkwort.

948. *P. Senega* L. Seneca Snakeroot. Frequent.
 949. *P. incarnata* L. Rare. Floyd and Emmet counties.
 950. *P. viridescens* L. (*P. sanguinea* L.) Common.
 951. *P. verticillata* L. Frequent on low prairies.

145. EUPHORBIACEAE (*Spurge Family*)**Croton** L.

952. *C. glandulosus* L. (var. *septrionalis* Muell.-Arg.) Infrequent in the southeastern part.
 953. *C. monanthogynus* Michx. Rare; Monona Co. (C. F. Balloun).
 954. *C. capitatus* Michx. Rare; Marion Co. (M. D. Textrum.) The species is widely distributed in the Southern States.

Acalypha L.

955. *A. virginica* L. Three-seeded Mercury. Common; woods and waste places.

Euphorbia L. Spurge.

956. *E. petaloidea* Engelm. (*Chamaesyce* Small). Rare; Story Co. (A. S. Hitchcock).
 957. *E. serpens* HBK. (*Chamaesyce* Small). Infrequent.
 958. *E. serpyllifolia* Pers. (*Chamaesyce* Small). Frequent in dry ground.
 959. *E. glyptosperma* Engelm. (*Chamaesyce* Small). Common.
 960. *E. Preslii* Guss. (*E. nutans* Lag., *Chamaesyce* Arthur) A common weedy species.
 961. *E. maculata* L. (*Chamaesyce* Small) Very common especially in pastures and cultivated ground.
 962. *E. hexagona* Nutt. (*Zygophyllidium* Small) Rare; Hardin and Boone counties. (L. H. Pammel)
 963. *E. marginata* Pursh. (*Dichrophyllum* Kl. & Garcke) Snow-on-the-Mountain. Frequent on our western border; also common in cultivation and as an escape.
 964. *E. corollata* L. (*Tithymalopsis* Kl. & Gareke) Very common in dry soil.

965. *E. heterophylla* L. (*Poinsettia* Small) Frequent in low ground.
 966. *E. obtusata* Pursh. (*Tithymalus* Kl. & Gareke) Rare; Webster and Decatur counties.
 967. *E. arkansana missouriensis* Norton. (*E. dictyosperma* of Gray's Manual, not of F. & M.; *Tithymalus arkansana* Kl. & Gareke) Rare; Humboldt, Webster and Johnson counties.
 *968. *E. Esula* L. (*Tithymalus* Hill.) Leafy Spurge. A troublesome perennial weed, becoming common. (Europe)
 *969. *E. lucida* Waldst. & Kit. (*Tithymalus* Kl. & Gareke) Infrequent; resembles the preceding. (Europe)
 *970. *E. Cyparissias* L. (*Tithymalus* Hill.) Cypress Spurge. Frequently planted in cemeteries and escaped to roadsides. (Europe)
 *971. *E. Peplus* L. (*Tithymalus* Hill.) Rare; Muscatine Co. (Europe)
 972. *E. commutata* Engelm. (*Tithymalus* Kl. & Gareke) Rare in the northeastern part.

146. CALLITRICHACEAE (*Water Starwort Family*)**Callitriche** L.

973. *C. palustris* L. Water Starwort. Rare; Emmet Co. (B. O. Wolden)
 974. *C. heterophylla* Pursh. Rare; Ringgold Co. (J. P. Anderson)

151. ANACARDIACEAE (*Sumac Family*)**Rhus** L.

975. *R. hirta* (L.) Sudworth. (*R. typhina* L.) Staghorn Sumac. Frequent northeast; rare farther west.
 976. *R. glabra* L. Smooth Sumac. Very common, border of woods.
 977. *R. copallina* L. Rare; Van Buren County. (L. H. Pammel)
 978. *R. Toxicodendron* L. (*Toxicodendron Toxicodendron* Britton; *T. Rydbergii* Greene) Poison Oak. The low, shrubby form, very common.
 979. *R. radicans* L. (*Toxicodendron* Kuntze) Poison Ivy; the climbing form. Infrequent, eastern and southern.
 980. *R. canadensis* Marsh. (*R. aromatica* Ait; *Schmaltzia crenata* Greene) Fragrant Sumac; frequent; also cultivated.

159. AQUIFOLIACEAE (*Holly Family*)**Ilex** L.

981. *I. verticillata* (L.) Gray. Black Alder or Winterberry, very rare; Mitchell County. (Mrs. H. F. Walker)

156. CELASTRACEAE (*Staff Tree Family*)**Euonymus** (Tourn.) L. (*Evonymus*)

982. *E. atropurpureus* Jacq. Burning Bush, Waahoo. Frequent in open woods; also cultivated.

Celastrus L.

983. *C. scandens* L. Climbing Bittersweet. Common; also cultivated. The vine when in mature fruit much used for winter decoration.

159. STAPHYLEACEAE (*Bladder Nut Family*)**Staphylea** L.

984. *S. trifolia* L. Bladder Nut. Frequent in open woods; a handsome shrub.

161. ACERACEAE (*Maple Family*)**Acer.** (Tourn.) L.

985. *A. spicatum* Lam. Mountain Maple. A shrubby species, frequent in open woods in the northeast section.
986. *A. saccharum* Marsh. (*A. subglaucum* Bush) Rock or Sugar Maple. Most common in the eastern part.
987. *A. nigrum* Michx. fil. (*A. saccharum* var. Britton.) Black Sugar Maple. This and the preceding valuable as shade trees, and their wood much used in cabinet work.
988. *A. saccharinum* L. Soft or Silver Maple. Common throughout the state. A rapid growing tree.
989. *A. Negundo* L. (*Negundo aceroides* Moench.) Box Elder. A very common and hardy tree, but not desirable for cultivation; includes several recently proposed segregates.

162. HIPPOCASTANACEAE (*Horse Chestnut Family*)**Æsculus** L. Horse Chestnut, Buckeye.

990. *Æ. glabra* Willd. Frequent in the southern half; also cultivated.
991. *Æ. glabra arguta* (Buckley) Robinson. Infrequent in the southern part.

166. BALSAMINACEAE (*Touch-me-not Family*)**Impatiens** (Rivinius) L. Balsam, Jewel-weed.

992. *I. pallida* Nutt. Pale Touch-me-not. Common in low, wet woods.
993. *I. biflora* Walt. (*I. fulva* Nutt.) Spotted Touch-me-not. Common in similar situations.

167. RHAMNACEAE (*Buckthorn Family*)**Rhamnus** (Tourn.) L. Buckthorn.

994. *R. alnifolia* L'Her. Rare in the eastern part.
- *995. *R. cathartica* L. Common Buckthorn. An occasional escape from cultivation. (Europe)
996. *R. lanceolata* Pursh. Most common in the southern half of the state.

168. VITACEAE (*Vine Family*)**Psedera** Neck.

997. *P. quinquefolia* (L.) Greene. (*Ampelopsis* Michx.; *Parthenocissus* Planchon) Virginia Creeper. Common; the tendrils mostly ending with adhesive disks.
998. *P. quinquefolia hirsuta* (Don) Rehder. a pubescent form. Infrequent.
999. *P. vitacea* (Knerr) Greene. Common; the leaves shining above, the tendrils with few or no adhesive disks.

Cissus L.

1000. *C. Ampelopsis* Pers. (*Ampelopsis cordata* Michx.) Rare in the northeast; Fremont Co. (J. P. Anderson, T. J. Fitzpatrick).

Vitis (Tourn.) L. Grape

1001. *V. aestivalis* Michx. Summer Grape; frequent in the northeastern section.
1002. *V. bicolor* Le Conte. Summer Grape. Rare in the northeast.

1003. *V. cinerea* Engelm. Sweet Winter Grape. Infrequent in the southern part.
 1004. *V. cordifolia* Michx. Frost Grape. Infrequent in the southern part.
 1005. *V. vulpina* L. (*V. riparia* Michx.) Common Wild Grape. River banks and open woods; introduced along fences by birds.
 1006. *V. rupestris* Scheele. Sand or Sugar Grape. Very rare, Lee Co. (L. H. Pammel) Perhaps introduced by birds from farther south.

172. TILIACEAE (*Linden Family*)**Tilia** (Tourn.) L.

1007. *T. americana* L. (*T. glabra* Vent.) American Linden or Basswood. Very common. A valuable shade tree; the flowers much visited by bees.

173. MALVACEAE (*Mallow Family*)**Abutilon** (Tourn.) Mill.

- *1008. *A. Theophrasti* Medic. Indian Mallow, Butter-print, Velvet-leaf. A common weed in fields and waste places, often difficult to eradicate. (Old World Tropics.)

Malvastrum Gray.

1009. *M. coccineum* (Pursh) Gray. (*Sphaeralcea* Rydb.) False Mallow. Rare, Harrison Co. (L. H. Pammel) Very common on the western plains.

Sida L.

- *1010. *S. spinosa* L. Indian or False Mallow. An Old World species, frequent in waste places. (Europe)

Althaea L.

- *1011. *A. rosea* Cav. Hollyhock. Frequently escaped. (China)

Malva (Tourn.) L.

- *1012. *M. rotundifolia* L. Common Mallow or Cheeses. Frequent as a weed near dwellings. (Europe).
 *1013. *M. verticillata* L. (Including *M. crispa* L.) Curled Mallow. Rare as an escape. (Europe)
 *1014. *M. sylvestris* L. High Mallow. Infrequent as an escape. (Europe)

Callirrhoe Nutt. Poppy Mallow.

1015. *C. triangulata* (Leavenw.) Gray. Rare in the southern part.
 1016. *C. involucrata* (T. & G.) Gray. Native in the southwestern part; also cultivated.

Napaea (Clayt.) L.

1017. *N. dioica* L. Glade Mallow. Infrequent in the eastern part.

Hibiscus L. Rose Mallow

1018. *H. militaris* Cav. Rare; river banks.
 *1019. *H. Trionum* L. Shoo-fly, Flower-of-an-Hour. Escaped from cultivation; a troublesome weed in some places. (Europe).

184. GUTTIFERACEAE (*Hypericaceae*)(*St. John's-wort Family*)**Hypericum** (Tourn.) L.

1020. *H. Ascyron* L. Great St. John's-wort. Frequent in dry ground.
 *1021. *H. perforatum* L. Common St. John's-wort. Our only introduced and weedy species; difficult to eradicate. (Europe).

- 1022. *H. punctatum* L. (*H. corymbosum* Muhl.) Common in low ground.
- 1023. *H. prolificum* L. Shrubby St. John's-wort. Infrequent.
- 1024. *H. cistifolium* Lam. (*H. sphaerocarpon* Michx.) Infrequent.
- 1025. *H. ellipticum* Hook. Rare in wet places.
- 1026. *H. mutilum* L. Frequent in our northern marshes.
- 1027. *H. majus* (Gray) Britton. (*H. canadense* var. Gray) Common in low places.
- 1028. *H. virginicum* L. (*Elodea campanulata* Pursh) Common in swamps.

Sarothra L.

- 1029. *S. gentianoides* L. (*Hypericum* BSP.) Orange-grass, Pine-weed. Rare in sandy soil. Clinton Co. (Fred Weiss)

190. CISTACEAE (*Rockrose Family*)

Helianthemum (Tourn.) Mill.

- 1030. *H. majus* (L.) BSP. (*Crocanthemum* Britton) Rockrose. Frequent in dry soil.

Lechea (Kalm) L. Pinweed.

- 1031. *L. minor* L. Infrequent in dry soil.
- 1032. *L. stricta* Leggett. Frequent.
- 1033. *L. tenuifolia* Michx. Frequent in dry soil.

195. VIOLACEAE (*Violet Family*)

Viola (Tourn.) L. Violet.

- 1034. *V. pedata* L. Bird-foot violet. Frequent on dry hillsides; a variable species.
- 1035. *V. pedata lineariloba* DC. Infrequent in dry soil.
- 1036. *V. cucullata* Ait. Common Blue violet. Flowers varying in color from white to dark blue; preferring low ground.
- 1037. *V. papilionacea* Pursh. Very common and resembling the preceding.
- 1038. *V. palmata* L. Infrequent. Leaves 5-9 lobed.
- 1039. *V. sororia* Willd. Infrequent. Moist soil in woods and on shady ledges.
- 1040. *V. sagittata* Ait. Rather rare in the eastern part.
- 1041. *V. pedatifida* G. Don. Common on dry prairies. Leaf forms variable.
- 1042. *V. scabriuscula* Schwein. (*V. eriocarpa* Schw.) Smooth Yellow Violet. Very common in woods. Typical *V. pubescens* with which this intergrades, may not occur within our limits.
- 1043. *V. canadensis* L. White violet. Frequent in woods.
- 1044. *V. conspersa* Reich. (*V. Muhlenbergii* Torr.) Rare; Winnesick and Allamakee counties.
- *1045. *V. tricolor* L. Our common Pansy; an occasional escape from cultivation. (Europe)

203. LOASACEAE (*Loasa Family*)

Nuttallia Raf.

- 1046. *N. decapetala* (Pursh) Greene. (*Mentzelia* Pursh) Showy *Mentzelia*. Infrequent on our northwestern border.

207. CACTACEAE (*Cactus Family*)**Opuntia** (Tourn.) Mill. Prickly Pear.

1047. *O. humifusa* Raf. (*O. Rafinesquii* Engelm.) Infrequent, sandy soil, limestone bluffs, Pottawattamie County. (L. H. Pammel), Lyon County. (R. I. Cratty)
1048. *O. fragilis* (Nutt.) Haw. Rare in very dry soil near Granite, Lyon Co. (L. H. Pammel)

211. THYMELAEACEAE (*Oleaster Family*)**Dirca** L.

1049. *D. palustris* L. Leatherwood, Moosewood, or Wicopy. Infrequent, eastern and southern. The tough, fibrous bark used by the Indians for thongs.

212. ELAEAGNACEAE (*Oleaster Family*)**Elaeagnus** (Tourn.) L.

- *1050. *E. angustifolia* L. Oleaster. Rare as an escape; Dickinson Co. (Europe)

Shepherdia Nutt.

1051. *S. argentea* Nutt. (*Lepargyrea* Greene) Buffalo Berry. Infrequent in the western part.

213. LYTHRACEAE (*Loosestrife Family*)**Didiplis** Raf.

1052. *D. diandra* (Nutt.) Wood. (*D. linearis* Raf.) Water Purslane. Rare; Ringgold and Decatur counties.

Rotala L.

1053. *R. ramosior* (L.) Koehne. (*Ammannia humilis* Michx.) Rare; Lee Co. ((Pammel & Reppert)

Ammannia (Houston) L.

1054. *A. coccinea* Rottb. Rare; Webster Co. (F. W. Paige)

Lythrum L.

1055. *L. alatum* Pursh. Loosestrife. Very common on low prairies.

Cuphea P. Br.

1056. *C. petiolata* (L.) Koehne. (*C. viscosissima* Jacq., *Parsonsia petiolata* Rusby) Rare; Lee Co. (P. H. Rolfs)

221. ONAGRASEAE (*Evening Primrose Family*)**Ludwigia** L. False Loosestrife.

1057. *L. alternifolia* L. Frequent.
1058. *L. polycarpa* Short & Peter. Frequent in wet places.

Chamaenerion (Tourn.) Adans.

1059. *C. angustifolium* (L.) Scop. (*Epilobium* L., *C. spicatum* S. F. Gray) Great Willow Herb or Fire-weed. Common in the north-eastern section.

Epilobium L. Willow Herb.

1060. *E. densum* Raf. (*E. lineare* Muhl.) Common in marshes.
1061. *E. coloratum* Muhl. Frequent in swampy places.
1062. *E. adenocaulon* Haussk. Common in similar habitats.

Oenothera L. Evening Primrose.

1063. *OE. muricata* L. Frequent, especially near timber; apt to be confused with the next.

1064. *OE. biennis* L. Common Evening Primrose. Very common and weedy in habit.
 1065. *OE. rhombipetala* Nutt. (*Raimannia* Rose) Frequent.
 *1066. *OE. laciniata* Hill. (*Raimannia* Rose) Rare; Polk Co. Recently introduced from Western Plains (Mrs. R. W. Zeuch; Lee Co., Jess L. Fults)
 *1067. *OE. Nuttallii* Sweet. (*Onagra* Spach) Rare, R.R. right-of-way, Story Co. (Western Plains)

Meriolix Raf.

1068. *M. serrulata* (Nutt.) Walp. (*Oenothera* Nutt.) Common on morainic hills in the northern part.

Gaura L.

1069. *G. biennis* L. Common; sometimes weedy in grain fields.
 1070. *G. coccinea* Pursh. Rare in dry soil in the western part.

Circaea (Tourn.) L. Enchanter's Nightshade.

1071. *C. lutetiana* L. Very common in woods.
 1072. *C. alpina* L. Rare in the eastern part; a much smaller plant.

222. HALORAGIDACEAE (*Water Milfoil Family*)**Myriophyllum** (Vaill.) L. Water Milfoil.

1073. *M. spicatum* L. Common in rather deep, quiet water.
 1074. *M. scabratum* Michx. (*M. pinnatum* BSP.) Infrequent in still water.

Proserpinaca L.

1075. *P. palustris* L. Mermaid Weed. Rare; Muscatine Co. (Ferd Reppert).

224. ARALIACEAE (*Ginseng Family*)**Aralia** (Tourn.) L.

1076. *A. racemosa* L. Spikenard. Common in rich woods.
 1077. *A. nudicaulis* L. Wild Sarsaparilla. Common in rich woods.

Panax L.

1078. *P. quinquefolium* L. Ginseng. Once common in rich woods, now becoming rare. The root valued as a cure-all by the Chinese.

225. UMBELLIFERACEAE (*Parsley Family*)**Eryngium** (Tourn.) L. Eringo.

1079. *E. yuccifolium* Michx. Rattle-snake Master. Button Snakeroot. Common.

Sanicula (Tourn.) L. Sanicle.

1080. *S. marilandica* L. Frequent in woods.
 1081. *S. gregaria* Bicknell. Common.
 1082. *S. canadensis* L. Frequent.

Chaerophyllum (Tourn.) L.

1083. *C. procumbens* (L.) Crantz. Infrequent in moist ground.

Osmorhiza Raf. Sweet Cicely.

1084. *O. Claytoni* (Michx.) Clarke. (*Washingtonia* Britton) Common in rich woods.
 1085. *O. longistylis* (Torr.) DC. (*Washingtonia* Britton) With the preceding and more common. The var. *villicaulis* Fernald also occurs.

Conium L.

- *1086. *C. maculatum* L. Poison Hemlock. Infrequent; an escape from gardens where it is cultivated under the misleading name of California Fern. The root a deadly poison. (Europe)

Cicuta L. Water Hemlock.

1087. *C. maculata* Spotted Cowbane. Very common in low ground; the tuberous roots very poisonous.
1088. *C. bulbifera* L. A more slender plant. Infrequent in swamps; the leaf axils bear numerous bulblets.

Carum L.

- *1089. *C. Carvi* L. Caraway. A common garden escape. (Europe)

Berula Hoffm.

1090. *B. erecta* (Huds.) Coville. Very rare. Emmet Co. (B. O. Wolden)

Sium (Tourn.) L.

1091. *S. cicutaefolium* Schrank. Common in wet places.

Cryptotaenia DC. (Deringa Adans.)

1092. *C. canadensis* (L.) DC. Honewort. Very common in open woods.

Zizia Koch.

1093. *Z. aurea* (L.) Koch. Golden Alexander. Common; resembles *Thaspium* but the central fruit in each umbel of this genus is sessile.
1094. *Z. cordata* (Walt.) DC. Frequent; preferring drier ground than the preceding.

Foeniculum (Tourn.) Hill.

- *1095. *F. vulgare* Hill. Cultivated and a frequent escape. (Europe)

Taenidia Drude.

1096. *T. integerrima* (L.) Drude. (*Pimpinella* Gray) Common in rich woods.

Thaspium Nutt. Meadow Parsnip.

1097. *T. trifoliatum* (L.) A. Gray. (*T. aureum* Nutt; *T. atropurpureum* Nutt.) Infrequent; open woods and prairies. Petals purple or yellow.
1098. *T. barbinode* (Michx.) Nutt. Frequent in similar habitats.

Cogswellia Spreng. (Lomatium Raf.)

1099. *C. orientalis* (C. & R.) (*Peucedanum nudicaule* Nutt. in part) Rare in dry ground. Emmet Co. (B. O. Wolden)

Pleiotaenia Coult. & Rose.

1100. *P. Nuttallii* (DC.) C. & R. (*Polytaenia* DC.) Infrequent in rather dry, barren soil.

Pastinaca L.

- *1101. *P. sativa* L. Parsnip; the common garden plant; a frequent escape as a weed in pastures and on roadsides. (Europe)

Heracleum L.

1102. *H. lanatum* Michx. Cow Parsnip. A common, coarse plant in rich woods.

Oxypolis Raf.

1103. *O. rigidior* (L.) C. & R. (*Tiedemannia* C. & R., *Archemora rigida* DC.) Frequent in low ground.

Falcaria Host.

- *1104. *F. vulgaris* Bernh. (*F. Rivini* Host.) Sicklewort. A recent introduction from Europe. Sioux Co. (Rex B. Conn) and Guthrie Co. (E. W. Gilman) A very persistent perennial. (Europe)

Torilis Adans.

- *1105. *T. Anthriscus* (L.) Bernh. Rare along a highway in Hardin Co. (L. H. Pammel) Introduced from Europe.

Daucus (Tourn.) L.

- *1106. *D. Carota* L. Wild Carrot. The garden cultivar; escaped as a weed to pastures and roadsides. (Europe)

226. CORNACEAE (*Dogwood Family*)**Cornus** (Tourn.) L. (*Svida Opiz*) Cornel, Dogwood.

1107. *C. rugosa* Lam. (*C. circinata* L'Her.) Round-leaved Dogwood. Frequent in woods.
 1108. *C. Amomum* Mill. Silky-leaved Dogwood, Kinnikinnik. Common.
 1109. *C. asperifolia* Michx. Rough-leaved Dogwood. Frequent. A rather tall shrub.
 1110. *C. stolonifera* Michx. Red-osier Dogwood. Infrequent except in the northern part.
 1111. *C. paniculata* L'Her. Common. Fruit white on bright red pedicils.
 1112. *C. alternifolia* L. fil. Common; the fruit deep blue in color.

228. PYROLACEAE (*Wintergreen Family*)**Pyrola** (Tourn.) L. Wintergreen, Shin-leaf.

1113. *P. secunda* L. Rare; dry woods and thickets.
 1114. *P. elliptica* Nutt. Frequent in woods.

Monotropa L.

1115. *M. uniflora* L. Indian Pipe. Quite rare in dark, rich woods.

Chimaphila Pursh.

1116. *C. umbellata* (L.) Nutt. Rare; Jackson and Fayette counties.

230. ERICACEAE (*Heath Family*)**Gaylussacia** HBK.

1117. *G. baccata* (Wang.) C. Koch. (*G. resinosa*. T. & G.) Black Huckleberry. Rare; Jones and Muscatine counties.

Vaccinium L. Blueberry, Huckleberry.

1118. *V. pennsylvanicum* Lam. Rare; Jones and Allamakee counties.
 1119. *V. canadense* Kalm. Velvet-leaf Blueberry. Rare; Clayton Co. (L. H. Pammel)

234. PRIMULACEAE (*Primrose Family*)**Primula** L.

1120. *P. mistassinica* Michx. Primrose. Very rare on limestone rocks near Iowa Falls, Hardin Co. (Blanche Stoddard)

Androsace (Tourn.) L.

1121. *A. occidentalis* Pursh. Rare in barren soil.

Lysimachia (Tourn.) L. Loosestrife.

1122. *L. terrestris* (L.) BSP. (*L. stricta* Ait.) Infrequent in low ground.

- *1123. *L. Nummularia* L. Moneywort. An occasional escape. (Europe)
 1124. *L. thyrsoiflora* L. Common, especially in northern marshes.

Steironema Raf.

1125. *S. ciliatum* (L.) Raf. Common in low ground.
 1126. *S. lanceolatum* (Walt.) Gray. (Including the var. *hybridum* Gray) Common.
 1127. *S. quadriflorum* (Sims) Hitchcock. (*S. longifolium* Gray) Common.

Dodecatheon L.

1128. *D. Meadia* L. Shooting Star. Frequent in the eastern and southern parts.

240. OLEACEAE (*Olive Family*)**Fraxinus** (Tourn.) L. Ash.

1129. *F. americana* L. White Ash. Common except in the northwestern part.
 1130. *F. pennsylvanica* Marsh. Frequent.
 1131. *F. lanceolata* Borkh. (*F. pennsylvanica* var. Sargent; *F. viridis* Michx. fil.) Green Ash. Our commonest species.
 1132. *F. quadrangulata* Michx. Blue Ash. Our rarest species. Des Moines Co. (L. H. Pammel)
 1133. *F. nigra* Marsh. Black Ash. Infrequent. Leaflets 7-11 and sessile.

243. GENTIANACEAE (*Gentian Family*)**Sabbatia** Adans. (*Sabatia*)

1134. *S. campestris* Nutt. Rare in clay soil; Jackson and Lee counties.

Gentiana (Tourn.) L. Gentian.

1135. *G. crinita* Froel. Fringed Gentian. Infrequent in low ground.
 1136. *G. procera* Holm. (*G. detonsa* Fries.) Rare; a narrow-leaved species.
 1137. *G. quinquefolia* L. (*G. quinqueflora* Hill.) Frequent in low ground.
 1138. *G. puberula* Michx. (*Dasystephana* Small) Common in dry prairie soil.
 1139. *G. Saponaria* L. (*Dasystephana* Small) Rare; Story Co. (R. I. Cratty) Perhaps overlooked because of its close resemblance to the next.
 1140. *G. Andrewsii* Griseb. (*Dasystephana* Small) Closed Gentian. A common large-flowered and late-blooming species. Probably the same as *G. clausa* Raf. If so, this latter name should be used as it antedates *G. Andrewsii* of Grisebach.
 1141. *G. flavida* Gray. (*Dasystephana* Small) White Gentian. Infrequent in woods.

Menyanthes (Tourn.) L.

1142. *M. trifoliata* L. Buckbean. Frequent in our northern marshes.

244. APOCYNACEAE (*Dogbane Family*)**Apocynum** (Tourn.) L. Dogbane.

1143. *A. androsaemifolium* L. Spreading Dogbane. Very common; usually near timber.
 1144. *A. medium* Greene. Rare; Lee Co. (Jess L. Fults)

- 1145. *A. cannabinum* L. Indian Hemp. Common and variable.
- 1146. *A. pubescens* R. Br. (*A. cannabinum* var. DC.) Rare; Decatur Co. (L. H. Pammel)
- 1147. *A. sibiricum* Jacq. (*A. cannabinum hypericifolium* (Ait.) Gray) Infrequent; Emmet and Decatur counties; the stem leaves with clasping bases.

245. ASCLEPIADACEAE (*Milkweed Family*)

Asclepias (Tourn.) L. Milkweed.

- 1148. *A. tuberosa* L. Pleurisy-root. Common in rather dry ground.
- 1149. *A. purpurascens* L. Frequent.
- 1150. *A. incarnata* L. Swamp Milkweed. Very common in low ground.
- 1151. *A. speciosa* Torr. Frequent on prairies in the northwestern part.
- 1152. *A. syriaca* L. (*A. Cornuti* Decn.) Common Milkweed or Silkweed. Very common.
- 1153. *A. Sullivantii* Engelm. Frequent on low prairies.
- 1154. *A. amplexicaulis* Smith. (*A. obtusifolia* Michx.) Rare; Winneshie Co. (E. W. Holway)
- 1155. *A. Meadii* Torr. Rare; Decatur Co. (J. P. Anderson)
- 1156. *A. phytolaccoides* Pursh. Infrequent.
- 1157. *A. ovalifolia* Decaisne. Frequent in dry ground.
- 1158. *A. quadrifolia* Jacq. Rare in the eastern part. Lower leaves whorled.
- 1159. *A. verticillata* L. Common; said to be poisonous to sheep.

Acerates Ell. Green Milkweed.

- 1160. *A. floridana* (Lam.) Hitch. Frequent.
- 1161. *A. viridiflora* Ell. Common and variable.
- 1162. *A. lanuginosa* (Nutt.) Decaisne. Rare in dry ground.

Gonolobus Michx.

- 1163. *G. laevis* Michx. Climbing Milkweed. Frequent in the southern part and spreading northward.

246. CONVOLVULACEAE (*Convolvulus Family*)

Breweria R. Br. (*Stylisma* Raf.)

- 1164. *B. Pickeringii* (M. A. Curtis) Gray. Rare; Muscatine Co. (L. H. Pammel)

Ipomoea L. Morning Glory

- *1165. *I. coccinea* L. Star Ipomoea. Rare as a garden escape. (Tropical Am.)
- 1168. *I. pandurata* (L.) G. F. W. Meyer. Wild Potato-vine, Man-of-the-Earth. Frequent in the southern part.
- 1169. *I. lacunosa* L. Rare; Muscatine Co. (J. H. Mackenzie)

Convolvulus (Tourn.) L. Bindweed.

- 1170. *C. spithameus* L. (*Calystegia* Gray) Infrequent; a low, usually upright plant in dry ground.
- *1171. *C. japonicus* Thunb. The so-called California Rose, a sterile garden cultivar, occasionally escaped by suckering. (Japan).
- 1172. *C. sepium* L. Wild Morning Glory or Hedge Bindweed. A very common and troublesome native plant in cultivated land.
- *1173. *C. arvensis* L. European Bindweed, Creeping Jenny. Frequent, and especially troublesome in the northwestern part. One of our unlawful weeds. (Europe).

Cuscuta (Tourn.) L. Dodder.

- *1174. *C. Epithymum* Murr. Occasional in clover fields; our only introduced species. (Europe).
- 1175. *C. Polygonorum* Engelm. (*C. obtusiflora* of Am. Authors not HBK.) Frequent on *Polygonum* and other herbs.
- 1176. *C. pentagona* Engelm. (*C. arvensis* of Am. Authors) Common on various herbs.
- 1177. *C. Coryli* Engelm. Hazel Dodder. Common on Hazel and other shrubs and tall herbs.
- 1178. *C. Cephalanthi* Engelm. Rare on shrubs and tall herbs.
- 1179. *C. Gronovii* Willd. Infrequent.
- 1180. *C. curta* (Engelm.) Rydb. Frequent; a large-fruited species.
- 1181. *C. glomerata* Choisy (*C. paradoxa* Raf.) A common rope-like parasite on tall herbs, mostly *Compositaceae*.

247. POLEMONIACEAE (*Polemonium* Family)**Phlox** L. Phlox.

- *1182. *P. paniculata* L. An escape from gardens with us. Native farther east and south.
- 1183. *P. maculata* L. Frequent in woods.
- 1184. *P. pilosa* L. Common, and extremely variable in color forms.
- 1185. *P. divaricata* L. Very common in low woods.
- 1186. *P. bifida* Beck. Rare; Blackhawk Co. (C. W. Lantz).
- *1187. *P. subulata* L. Rare as an escape around old gardens. (Atlantic States).

Gilia Ruiz & Pavon.

- 1188. *G. linearis* (Nutt.) Gray. Rare as a native, Dickinson Co. H. S. Conard), and occasionally introduced.

Navarretia Ruiz & Pavon.

- *1189. *N. intertexta* Hook. (*Gilia* Benth.) A rare introduction from the far west, Page Co. (E. H. Eichling).

Polemonium (Tourn.) L.

- 1190. *P. reptans* L. Greek Valerian, Jacob's Ladder. Frequent; the specific name is misleading, the plant being upright or spreading.

248. HYDROPHYLLACEAE (*Waterleaf* Family)**Hydrophyllum** (Tourn.) L. Waterleaf.

- 1191. *H. virginianum* L. Very common in rich woods.
- 1192. *H. appendiculatum* Michx. Frequent in the eastern part.

Ellisia L.

- 1193. *E. Nyctelea* L. Very common in low woods; one of our earliest spring flowers.

249. BORAGINACEAE (*Borage* Family)**Cynoglossum** (Tourn.) L.

- *1194. *C. officinale* L. Hound's Tongue. Frequent in waste places. (Europe).

Lappula (Rivini.) Moench.

- 1195. *L. virginiana* (L.) Greene. (*Echinosperrum* Lehm.) Beggar's Lice. Very common in woods; a most disagreeable weed.

- *1196. *L. echinata* Gilibert. (*Echinosperrum Lappula* Lehm.) Frequent in waste places. (Europe).
1197. *E. Redowskii occidentalis* (S. Wats.) Rydb. (*L. texana* Britton) Rare in the northeastern part.
- Myosotis** (Rupp.) L. Forget-me-not.
1198. *M. virginica* (L.) BSP. (*M. verna* Nutt.) Very rare; Garrison Rock, Ottumwa, Wapello Co. (L. H. Pammel).
- Mertensia** Roth. Lungwort.
1199. *M. virginica* (L.) Link. Virginian Cowslip. Frequent. White-flowered forms occur rarely.
1200. *M. paniculata* (Ait.) G. Don. Infrequent in the northeast.
- Lithospermum** (Tourn.) L. Gromwell, Puccoon.
1201. *L. latifolium* Michx. Frequent in woods.
1202. *L. Gmelini* (Michx.) Hitch. (*L. hirtum* Lehm.) Infrequent.
1203. *L. canescens* (Michx.) Lehm. Puccoon, Indian Paint-root. Very common; prairies and open woods.
1204. *L. linearifolium* Goldie. (*L. angustifolium* Michx.) Frequent in dry ground. The early flowers are large and conspicuous. Later the plant is diffusely branched with small cleistogamous flowers which are very fertile.
- Onosmodium** Michx.
1205. *O. occidentale* Mackenzie. False Gromwell. Common in dry ground.
- Echium** (Tourn.) L.
- *1206. *E. vulgare* L. Viper's Bugloss, Blue-weed. Infrequent. (Europe).
250. VERBENACEAE (*Vervian Family*)
- Verbena** (Tourn.) L. Verbena, Vervain.
1207. *V. urticaefolia* L. A common, white-flowered species.
1208. *V. angustifolia* Michx. Infrequent.
1209. *V. stricta* Vent. A common, weedy species, especially in pastures.
1210. *V. hastata* L. Very common.
1211. *V. bracteosa* Michx. Prostrate Vervain. Common in dry ground, especially near dwellings. All our native species of *Verbena* hybridize freely.
- *1212. *V. hybrida*, the garden cultivar is an occasional escape—probably a fusion of two or more tropical species.
- Lippia** (Houston) L.
1213. *L. lanceolata* Michx. (*Phyla* Greene) Fog-fruit. Frequent in sandy soil.
251. LABIATACEAE (*Mint Family*)
- Teucrium** (Tourn.) L. Germander.
1214. *T. canadense* L. Common, especially in or near woods.
1215. *T. occidentale* Gray. Common in low, swampy ground.
- Isanthus** Michx.
1216. *I. brachiatus* (L.) BSP. (*I. coeruleus* Michx.) False Penny-royal. Infrequent.

Scutellaria L. Skulleap.

1217. *S. lateriflora* L. Mad-dog Skulleap. Common along streams.
 1218. *S. ovata* Hill. (*S. versicolor* of Am. Authors) Frequent; moist banks.
 1219. *S. epilobifolia* Hamilton. (*S. galericulata* of Am. Authors not L.). Common in marshes.
 1220. *S. parvula* Michx. The typical plant rare southeast.
 1221. *S. ambigua* Nutt. (*S. parvula* var. Fern.) Common in dry soil; woods and prairies.

Marrubium (Tourn.) L.

- *1222. *M. vulgare* L. Common Horehound. Frequent as a naturalized plant. (Europe).

Agastache Clayt. Giant Hyssop.

1223. *A. nepetoides* (L.) Kuntze. Common.
 1224. *A. scrophulariaefolius* (Willd.) Kuntze. Very common. The var. *mollis* Fernald occurs in Decatur Co. (J. P. Anderson).
 1225. *A. Foeniculum* (Pursh) Kuntze. Rare in dry wooded ravines; Emmet Co. (R. I. Cratty, B. O. Wolden).

Nepeta L.

- *1226. *N. Cataria* L. Catnip. Very commonly introduced. (Europe).
 *1227. *N. hederacea* (L.) Trevisan. (*Glechoma* Benth.) Ground Ivy, Gill-over-the-Ground. Frequent. (Europe).

Dracocephalum (Tourn.) L.

1228. *D. parviflorum* Nutt. (*Moldavica* Britton.) Dragon-head. Infrequent.

Prunella L.

1229. *P. vulgaris* L. Self-heal. Common; also native of Europe.

Physostegia Benth.

1230. *P. parviflora* Nutt. (*Dracocephalum Nuttallii* Britton). Smaller False Dragon-head. Common near water. This was included in *P. virginiana* in the older manuals.

Galeopsis L.

- *1231. *G. Tetrahit* L. Hemp Nettle. Infrequent in waste places. (Europe).

Lamium L.

- *1232. *L. amplexicaule* L. Henbit. Infrequent in waste places. (Europe).

Leonurus L.

- *1233. *L. Cardiaca* L. Motherwort; a common, disagreeable weed. (Europe).

Stachys (Tourn.) L. Hedge Nettle.

1234. *S. ambigua* (Gray) Britton. Rare.
 1235. *S. tenuifolia* Willd. (*S. aspera glabra* Gray) Infrequent.
 1236. *S. aspera* Michx. (*S. tenuifolia* var. Fernald) Frequent in low ground.
 1237. *S. palustris* L. (*S. scopulorum* Greene). Common.
 1238. *S. palustris homotricha* Fernald (*S. homotricha* Rydb.) Frequent.
 1239. *S. pustulata* Rydb. Infrequent.
 1240. *S. Schweinitzii* Rydb. (*S. velutina* Schwein.) Frequent.

Salvia (Tourn.) L. Sage.

- *1241. *S. silvestris* L. Rare; Dickinson and Pottawattamie counties. (Europe).
- 1242. *S. lanceolata* Willd. (*S. lanceaefolia* Auth. not Poir.) Infrequent as a native, but spreading rapidly.

Monarda L. Horse Mint.

- 1243. *M. didyma* L. Oswego Tea, Bee Balm. Rare near the eastern border; perhaps introduced.
- 1244. *M. mollis* L. Very common. The eastern *M. fistulosa* may be found in the state.
- 1245. *M. punctata* L. Frequent in the eastern part.

Blephilia Raf.

- 1246. *B. hirsuta* (Pursh) Benth. Torr. Frequent.

Hedeoma Pers. Mock Pennyroyal.

- 1247. *H. pulegioides* (L.) Pers. Frequent.
- 1248. *H. hispida* Pursh. Common, especially in dry ground.

Pycnanthemum Michx. Mountain Mint.

- 1249. *P. flexuosum* (Walt.) BSP. Frequent.
- 1250. *P. virginianum* (L.) Durand & Jackson. Very common.
- 1251. *P. pilosum* Nutt. (*P. muticum* var. Gray) Infrequent.

Lycopus (Tourn.) L. Water Horehound.

- 1252. *L. virginicus* L. Very common in marshes.
- 1253. *L. uniflorus* Michx. Infrequent.
- 1254. *L. rubellus* Moench. Frequent in low ground.
- 1255. *L. asper* Greene. (*L. lucidus americanus* Gray). Infrequent.
- 1256. *L. americanus* Muhl. (*L. sinuatus* Ell.). Common in low ground.

Mentha (Tourn.) L. Mint.

- *1257. *M. spicata* L. Spearmint. Infrequent as an escape.
- *1258. *M. piperita* L. Peppermint. Rare as an escape; Decatur Co. (Europe).
- *1259. *M. gentilis* L. Frequent as an introduced plant. (Europe).
- 1260. *M. arvensis canadensis* (L.) Briquet. Wild Peppermint. Very common.

253. SOLANACEAE (*Nightshade Family*)**Solanum** (Tourn.) L. Nightshade.

- *1261. *S. Dulcamara* L. Bittersweet. A frequent escape from cultivation. (Europe).
- *1262. *S. triflorum* Nutt. Rare; native west and southwest.
- 1263. *S. nigrum* L. Black Nightshade. Common, especially in cultivated ground.
- *1264. *S. carolinense* L. Horse Nettle. A common, noxious, perennial weed. (s.e. U. S.).
- *1265. *S. rostratum* Dunal. Buffalo Bur. An immigrant from the western plains.
- *1266. *S. citrullifolium* A. Br. Rare; Fayette Co. (s.w. U. S.).
- *1267. *S. sisymbriifolium* Lam. Very rare; Fairfield, Jefferson Co. (Tropical Am.)

Physalis L. Ground Cherry.

- *1268. *P. ixocarpa* Brotero. The Tomentilla. Rare as an escape; Dickinson Co. (s.w. U. S.).
- *1269. *P. pubescens* L. Ground Cherry. Cultivated and escaped. (s.e. U. S.).
- 1270. *P. pruinosa* L. Rare southeast.
- 1271. *P. missouriensis* Mackenzie. Infrequent.
- 1272. *P. subglabrata* Mack. & Bush. Frequent.
- *1273. *P. Alkekengi* L. Chinese Lantern. Cultivated and occasionally escaped; the roots stoloniferous. (China).
- 1274. *P. heterophylla* Nees. Common.
- 1275. *P. heterophylla ambigua* (Gray) Rydb. Less frequent than the species.
- 1276. *P. longifolia* Nutt. Frequent.
- 1277. *P. macrophysa* Rydb. Rare; Emmet Co. (B. O. Wolden)
- 1278. *P. pumila* Nutt. Rare; Story Co. (fide Dr. Rydberg)
- 1279. *P. virginiana* Mill. Frequent. An erect, perennial species.
- 1280. *P. lanceolata* Michx. Frequent in dry soil.

Lycium L.

- *1281. *P. halimifolium* Mill. (*L. vulgare* Dunal) Matrimony Vine. A frequent escape. (Europe).

Datura L. Jimson Weed.

- *1282. *D. Stramonium* L. An infrequent, rank-smelling weed. (Asia).
- *1283. *D. Tatula* L. Purple-stemmed Jimson Weed. Rare; perhaps only a form of the preceding. (Asia).

254. SCROPHULARIACEAE (*Figwort Family*)**Verbascum (Tourn.) L.** Mullein.

- *1284. *V. Thapsus* L. Common Mullein. Frequent. (Europe).
- *1285. *V. Blattaria* L. Moth Mullein. Infrequent. (Europe).

Linaria (Tourn.) Hill. Toadflax.

- *1286. *L. vulgaris* Hill. Ramsted, Butter and Eggs. Cultivated and a common escape. (Europe).
- 1287. *L. canadensis* (L.) Dumont. Rare in sandy soil.
- *1288. *L. minor* (L.) Desf. (*Chaenorhinum* Pennell) Rare; Chickasaw Co. (Europe).

Antirrhinum (Tourn.) L.

- *1289. *A. majus* L. Snapdragon. Rare as a garden escape. (Europe).

Scrophularia (Tourn.) L. Figwort.

- 1290. *S. marilandica* L. (*S. nodosa* var. Gray) The typical form infrequent.
- 1291. *S. marilandica* f. *neglecta* (Rydb.) Pennell. Common.
- 1292. *S. lanceolata* Pursh. (*S. leporella* Bicknell.) Frequent in woods.

Pentstemon (Mitchell) Ait. Beard-tongue.

- 1293. *P. pallidus* Small. (*P. hirsutus* of Gray's Manual in part, not Willd.) Infrequent; southern and eastern.
- 1294. *P. Digitalis* (Sweet.) Nutt. Rare as a native; also cultivated.
- 1295. *P. grandiflorus* Nutt. Infrequent in dry ground.

Chelone (Tourn.) L. Turtle-head.

- 1296. *C. glabra* L. Frequent.

1297. *C. obliqua speciosa* Pennell & Wherry. Purple-flowered Turtle-head. Rare; Mitchell Co. (F. May Tuttle) and Lee Co. (Paul Bartsch)
 1298. *C. linifolia* f. *velutina* Pennell & Wherry. Rare; a very narrow-leaved form.

Mimulus L. Monkey Flower.

1299. *M. ringens* L. Common in marshes.
 1300. *M. alatus* Ait. Rare; Decatur Co. (J. P. Anderson)
 1301. *M. glabratus Fremontii* (Benth.) Pennell. (*M. glabratus Jamesii* Gray) Rare in water or wet places.

Conoclea Aublet.

1302. *C. multifida* (Michx.) Benth. (*Leucospora* Pennell) Infrequent.

Bacopa Aublet.

1303. *B. rotundifolia* (Michx.) Wetts. (*Herpestis* Pursh; *Monniera* Michx.) Water Hyssop. Rare; Fremont Co. (A. S. Hitchcock).

Gratiola L.

1304. *G. neglecta* Torr. (*G. virginiana* in part) Common in low ground.

Ilysanthes Raf. False Pimpernel.

1305. *I. dubia* (L.) Barnhart. Infrequent.
 1306. *I. dubia riparia* (Raf.) Pennell. Frequent on wet banks and low prairies.
 1307. *I. inaequalis* (Walt.) Pennell. Very rare; Mitchell Co. (F. May Tuttle).

Veronica (Tourn.) L. Speedwell.

1308. *V. Anagallis-aquatica* L. Water Speedwell. Frequent north-east.
 1309. *V. americana* Schwein. Rare; Lee Co. (A. S. Hitchcock).
 1310. *V. catenata* Pennell. Winneshiek, Hamilton and Story counties.
 1311. *V. catenata glandulosa* (Farwell) Pennell. Infrequent in the central part.
 1312. *V. scutellata* L. Rare; Buchanan Co. (Ex. Herb. C. E. Bessey).
 1313. *V. serpyllifolia* L. Rare; Chickasaw Co. (W. D. Spiker).
 1314. *V. peregrina* L. A common weed.
 *1315. *V. arvensis* L. Frequent. (Europe).
 *1316. *V. persica* Poir. (*V. Tournefortii* C. C. Gmelin) Rare; Chickasaw Co. (Europe).

Veronicastrum Moench.

1317. *V. virginicum* (L.) Pennell. (*Veronica* L.) Culver's Root. Very common; prairies and open woods.

Synthyris Benth.

1318. *S. Bullii* (Eaton) Heller. (*Besseya* Rydb.) Infrequent; northern and eastern.

Agalinis Raf.

1319. *A. aspera* (Michx.) Britton. (*Gerardia aspera* Dougl.) Common on upland prairies.
 1320. *A. purpurea* (L.) Britton. (*Gerardia* L.) Frequent on low prairies.

1321. *A. Gattingeri* (Small) Pennell. (*Gerardia* Small) Rare; Decatur Co. (J. P. Anderson).
 1322. *A. Besseyana* Britton. (*G. tenuifolia macrophylla* Benth.) Frequent.
 1323. *A. tenuifolia parviflora* (Nutt.) Pennell. Our commonest narrow-leaved form.

Otophylla Benth.

1324. *O. auriculata* (Michx.) Small. (*Tomanthera* Pennell; *Gerardia* Michx.) Frequent in low ground.

Aureolaria Raf.

1325. *A. grandiflora pulchra* Pennell. (*Gerardia grandiflora* Benth.) Frequent in the eastern counties.

Dasystoma Raf. Foxglove.

1326. *D. macrophylla* (Nutt.) Raf. (*Seymeria* Nutt.; *Afzelia* Kuntze; *Gerardia* Benth.) Western Foxglove; infrequent.

Castilleja Mutis. Painted Cup.

1327. *C. coccinea* (L.) Spreng. Frequent in open woods.
 1328. *C. sessiliflora* Pursh. Frequent on dry hillsides, especially northward.

Pedicularis (Tourn.) L. Lousewort.

1329. *P. canadensis* L. Common on low prairies, and in open woods.
 1330. *P. lanceolata* Michx. Frequent in wet ground.

255. BIGNONIACEAE (*Bignonia* Family)**Tecoma** Juss.

1331. *T. radicans* (L.) Juss. Trumpet Flower. Infrequent as a native in the southern part, but common in cultivation.

257. MARTYNIACEAE (*Martynia* Family)**Martynia** L.

1332. *M. louisiana* Mill. (*M. proboscidea* Gloxin.) Unicorn plant. Infrequent in the southern part; also occasional in cultivation.

258. OROBANCHACEAE (*Broom-rape* Family)**Orobanche** (Tourn.) L. Broom-rape.

1333. *O. uniflora* L. (*Aphyllon* Gray; *Thalesia* Britton) Infrequent on low prairies.
 1334. *O. fasciculata* Nutt. (*Aphyllon* Gray; *Thalesia* Britton) Rare; Dickinson Co. (A. S. Hitchcock).

261. LENTIBULARIACEAE (*Bladderwort* Family)**Utricularia** L. Bladderwort.

1335. *U. macrorrhiza* Le Conte (*U. vulgaris americana* Gray) Common in shallow, grassy ponds.
 1336. *U. minor* L. Very rare; Emmet Co. (R. I. Cratty).
 1337. *U. intermedia* Hayne. Infrequent in northern marshes.

263. ACANTHACEAE (*Acanthus* Family)**Ruellia** (Plumier) L.

1338. *R. ciliosa* Pursh. Infrequent in dry soil.
 1339. *R. strepens* L. Infrequent in dry woods.

Dianthera (Gronov.) L.

1340. *D. americana* L. Water Willow. Rare; growing in water. Lee Co. (Jess L. Fufts).

265. **PHRYMACEAE** (*Lop-seed Family*)**Phryma** L.

1341. *P. Leptostachya* L. Lop-seed. Very common in woods.

266. **PLANTAGINACEAE** (*Plantain Family*)**Plantago** (Tourn.) L. Plantain.

- *1342. *P. major* L. Very common. Native in the far north and in Eurasia.
 1343. *P. Rugelii* Deen. Common.
 *1344. *P. eriopoda* Torr. Rare; adventive on R.R. right-of-way, Story Co. (J. P. Anderson) (Western Plains).
 *1345. *P. lanceolata* L. Buckhorn, Rib-grass. A common noxious weed in lawns and pastures. (Europe).
 1346. *P. Purshii* R. & S. Frequent in dry, sandy soil.
 1347. *P. aristata* Michx. Bracted Plantain. Frequent in dry ground.
 1348. *P. virginica* L. Infrequent.

267. **RUBIACEAE** (*Madder Family*)**Galium** L. Bedstraw.

1349. *G. Aparine* L. Very common; woods and thickets.
 *1350. *G. Verum* L. Yellow Bedstraw. Infrequent; Cass Co. (Europe).
 1351. *G. circaezans* Michx. Frequent.
 1352. *G. boreale* L. Northern Bedstraw. Common; open prairies and near water.
 1353. *G. trifidum* L. (*G. trifidum pusillum* Gray) Frequent; bogs and mossy woods.
 1354. *G. Claytoni* Michx. Frequent in low ground, often in grassy places.
 1355. *G. tinctorium* L. (*G. trifidum latifolium* Torr.) Common.
 1356. *G. concinnum* T. & G. Common in rich woods.
 1357. *G. asprellum* Michx. Infrequent.
 1358. *G. triflorum* Michx. Sweet-scented Bedstraw. Frequent in rich woods.

Diodia (Gronov.) L.

- *1359. *D. teres* Walt. Button-weed. Adventive on the I. S. C. Campus, Story Co. (C. C. Lounsberry) (Southern U. S.).

Cephalanthus L.

1360. *C. occidentalis* L. Button-bush. Infrequent; usually near streams.

Houstonia L. Bluets, Innocence.

1361. *H. minima* Beek. Rare; Johnson and Buchanan counties.
 1362. *H. angustifolia* Michx. Infrequent in sterile soil.

268. **CAPRIFOLIACEAE** (*Honeysuckle Family*)**Diervilla** (Tourn.) L.

1363. *D. Lonicera* Mill. (*D. trifida* Moench). Bush Honeysuckle. Frequent.

Lonicera L. Honeysuckle.

- *1364. *L. tatarica* L. (*Xylosteon* Medic.) Tartarian Honeysuckle. Very common in cultivation and an occasional escape. (Asia).

- *1365. *L. sempervirens* L. Rare as an escape. Chickasaw Co. (s.e. U. S.).
- 1366. *L. prolifera* (Kirchner) Rehder. (*L. Sullivantii* Gray) Frequent in the northeastern section.
- 1367. *L. dioica* L. (*L. glauca* Hill.) A common, bushy species in open woods.
- 1368. *L. glaucescens* Rydb. Frequent. A segregate from the preceding.

Symphoricarpos (Dill.) Ludwig.

- 1369. *S. orbiculatus* Moench. Coral-berry. Frequent as a native; also cultivated.
- 1370. *S. occidentalis* Hook. Wolf-berry, Buck-brush. Common; open woods and prairies.
- *1371. *S. racemosus laevigatus* Fernald. The Snow-berry of the gardens. Rare as an escape. (n.e. U. S.).

Linnaea (Gronov.) L. Twin-flower.

- 1372. *L. borealis americana* (Forbes) Rehder. Rare in mossy woods. Northeastern section.

Triosteum L. Horse Gentian.

- 1373. *T. perfoliatum* L. Common; open woods and banks of streams.
- 1374. *T. aurantiacum* Bicknell. Frequent. Mature fruit bright orange-red.

Viburnum (Tourn.) L. Arrow-wood.

- 1375. *V. trilobum* Marsh. (*V. Opulus americanum* Ait.) Infrequent in the northeastern part.
- 1376. *V. affine* Bush. (*V. pubescens* of Authors in part) Frequent, especially northward.
- 1377. *V. affine hypomalacum* Blake. Infrequent.
- 1378. *V. molle* Michx. Rare; Oakland Mills, Pottawattamie Co. (L. H. Pammel).
- 1379. *V. dentatum* L. Rare; Decatur Co. (J. P. Anderson; Lee Co., Jess L. Fults).
- 1380. *V. Lentago* L. Black Haw. A common species in open woods; the fruit edible.
- 1381. *V. prunifolium* L. Infrequent on wooded ledges. Lee Co. (Cratty and Fults).

269. ADOXACEAE (*Moschatel Family*)

Adoxa L.

- 1382. *A. Moschatellina* L. Moschatel. Rare; Winneshiek, Fremont and Floyd counties; about its southern limit in the U. S.; also in northern Eurasia.

270. VALERIANACEAE (*Valerian Family*)

Valeriana (Tourn.) L.

- 1383. *V. edulis* Nutt. Valerian. Infrequent; the root sometimes cooked for food.

272. CUCURBITACEAE (*Gourd Family*)

Sicyos L.

- 1384. *S. angulatus* L. One-seeded Bur-cucumber. Infrequent; mostly in the northeastern section.

Micrampelis Raf.

1385. *M. lobata* (T. & G.) Greene. (*Echinocystis* T. & G.) Willd. Balsam Apple, Cucumber-vine. Common in or near timber, climbing over bushes.

273. CAMPANULACEAE (*Bluebell Family*)**Specularia** (Heist.) Fabricius.

1386. *S. perfoliata* L. Venus's Looking-glass. Frequent in woods.

Campanula (Tourn.) L. Bellflower.

1387. *C. americana* L. (*Campanulastrum* Rydb.) Common in open woods.
 1388. *C. rotundifolia* L. Harebell. Common and variable.
 1389. *C. aparinoides* Pursh. Marsh Harebell. Frequent in marshes.
 1390. *C. uliginosa* Rydb. Infrequent in wet soil, banks of streams.

Lobelia (Plumier) L. Lobelia.

1391. *L. cardinalis* L. Cardinal Flower. Frequent in open woods.
 1392. *L. siphilitica* L. Great Lobelia. Common in low ground.
 1393. *L. spicata* Lam. Common and extremely variable.
 1394. *L. Kalmii* L. Rare in the northern part.
 1395. *L. inflata* L. Indian Tobacco. A common woodland species.

277. COMPOSITACEAE (*Composite Family*)**Vernonia** Schreb. Ironweed.

1396. *V. fasciculata* Michx. Very common in low ground.
 1397. *V. altissima* Nutt. (*V. maxima* Small) Infrequent; Lee Co. (Jess L. Fults).
 1398. *V. missourica* Raf. Infrequent.
 1399. *V. interior* Small. (*V. Baldwinii* of Gray's Man. in part) Frequent in the southeast.

Eupatorium (Tourn.) L. Thoroughwort.

1400. *E. Holzingeri* Rydb. Infrequent; Webster and Fayette counties.
 1401. *E. falcatum* Michx. Infrequent in the central part.
 1402. *E. maculatum* L. Common.
 1403. *E. purpureum* L. Common.
 1404. *E. Bruneri* Gray. Rare.
 1405. *E. perfoliatum* L. Boneset. Common in low ground.
 1406. *E. altissimum* L. Frequent.
 1407. *E. serotinum* Michx. Infrequent; resembles the next.
 1408. *E. urticaefolium* Reichard. White Snakeroot. Very common in woods. Said to be poisonous to stock.

Kuhnia L.

1409. *K. eupatorioides* L. (*K. glutinosa* Ell.) Common in dry ground.

Liatris Schreb. Button Snakeroot, Blazing Star.

1410. *L. squarrosa* Willd. (*Laciniaria* Hill). Infrequent.
 1411. *L. cylindracea* Michx. (*Laciniaria* Kuntze). Infrequent in dry ground.
 1412. *L. punctata* Hook. (*Laciniaria* Kuntze). Frequent on moraine hills, especially northward.
 1413. *L. scariosa* Willd. (*Laciniaria* Hill). Common and variable. The var. *racemulosa* Sheldon is an ecological form with the heads long-peduncled.
 1414. *L. pycnostachya* Michx. (*Lacinaria* Kuntze). Very common.

Grindelia Willd.

1415. *G. squarrosa* (Pursh.) Dunal. Gum-plant. Becoming common as a weed.

Chrysopsis Nutt. Golden Aster.

1416. *C. angustifolia* Rydb. Rare in very dry ground. Lyon Co. (L. H. Pammel).
 1417. *C. villosa* Nutt. Rare; Clinton Co. (L. H. Pammel).

Solidago L. Golden-rod.

1418. *S. latifolia* L. Frequent in or near timber.
 1419. *S. hispida* Muhl. (*S. bicolor concolor* T. & G.) Rare.
 1420. *S. speciosa* Nutt. Infrequent in open woods.
 1421. *S. speciosa angustata* T. & G. (*S. rigidiuscula* Porter). Frequent in dry ground.
 1422. *S. ulmifolia* Muhl. Frequent in open woods.
 1423. *S. glaberrima* Mart. (*S. missouriensis* of Gray's Manual, not Nutt.) Our commonest early-flowering species; late flowering and variable forms are occasionally seen.
 1424. *S. nemoralis* Ait. Very common and variable. Large-flowered forms approach *S. pulcherrima* Aven Nelson.
 1425. *S. longipetiolata* Mackenzie. Infrequent. A recent segregate from the preceding.
 1426. *S. canadensis* L. Very common and variable.
 1427. *S. serotina* Ait. Very common in low ground.

Oligoneuron Small.

1428. *O. rigidum* (L.) Small. (*Solidago* L.) Very common in dry ground.
 1429. *O. Riddellii* (Frank) Small. (*Solidago* Frank) Infrequent on low prairies.

Euthamia Nutt.

1430. *E. graminifolia* (L.) Nutt. (*Solidago* Salisb.) Common on low prairies.

Sideranthus Nutt. Iron Plant.

1431. *S. spinulosus* (Pursh) Sweet. (*Aplopappus* DC.; *Eriocarpum* Greene) Frequent in the western part.

Boltonia L'Her.

1432. *B. asteroides* (L.) L'Her. Common on low prairies or in swampy ground. Occasional in cultivation.

Brachyactis Ledeb.

1433. *B. angusta* (Lindl.) Britton. (*Aster* T. & G.) Rare in low ground. Emmet Co. (R. I. Cratty).

Aster (Tourn.) L. Aster.

1434. *A. oblongifolius* Nutt. Frequent.
 1435. *A. novae-angliae* L. New England Aster. A common and handsome species.
 1436. *A. sericeus* Vent. Silky-leaved Aster. Common in dry ground.
 1437. *A. azureus* Lindl. Frequent; a handsome species.
 1438. *A. Shortii* Lindl. Infrequent in the southern part.
 1439. *A. cordifolius* L. Frequent in woods.
 1440. *A. Finkii* Rydb. Rare; type No. 83,629 I.S.C. Herb. Fayette Co. (Bruce Fink).

- 1441. *A. sagittifolius* Wedemeyer. Very common in open woods.
- 1442. *A. Drummondii* Lindl. Frequent in open woods. This and the three preceding form an extremely variable group.
- 1443. *A. laevis* L. Smooth Aster. Common.
- 1444. *A. concinnus* Willd. Rare; Emmet Co. (F. W. Paige, R. I. Cratty).
- 1445. *A. ericoides* L. Most frequent in the southeastern part.
- 1446. *A. multiflorus* Ait. Common on dry prairies and roadsides.
- 1447. *A. multiflorus exiguus* Fernald. (*A. exiguus* Rydb.) Frequent. Heads single or slightly clustered on drooping pedicels.
- 1448. *A. lateriflorus* (L.) Britton. (*A. diffusus* Ait.) Common; thickets and open woods; extremely variable.
- 1449. *A. Tradescanti* L. Frequent.
- 1450. *A. paniculatus* Lam. Frequent in low ground.
- 1451. *A. salicifolius* Ait. Frequent on low prairies.
- 1452. *A. junceus* Ait. Rare in bogs. Emmet Co. (B. O. Wolden)
- 1453. *A. nebraskensis* Britton. Rare in boggy places. Emmet and Marshall counties; resembles *A. puniceus*.
- 1454. *A. Woldeni* Rydb. Rare in dry ground. Ellsworth Tp. Emmet Co. (B. O. Wolden). Co-type in I.S.C. Herb. No. 116,905. The type in the herbarium of the New York Botanic Garden.
- 1455. *A. prenanthoides* Muhl. Frequent in or near timber; the stem with pubescent lines.
- 1456. *A. puniceus* L. Frequent; a beautiful species.
- 1457. *A. ptarmicoides* T. & G. (*Doellingeria* Nees.) Infrequent in dry soil.
- 1458. *A. umbellatus* Mill. (*Diplopappus* Hook.; *Doellingeria* Nees.) Infrequent in wet soil.

Ionactis Greene.

- 1459. *I. linariifolia* (L.) Greene. (*Aster linariifolius* (L.) A beautiful aster-like plant, rare in dry ground. Grand Mound, Clinton Co. (Fred Weiss).

Erigeron L. Fleabane.

- 1460. *E. philadelphicus* L. Common.
- 1461. *E. annuus* (L.) Pers. Daisy Fleabane.
- 1462. *E. ramosus* (Walt.) BSP. Common.

Leptilon Raf.

- 1463. *L. canadensis* (L.) Britton. (*Erigeron* L.) Horseweed. Common in cultivated ground and waste places.
- 1464. *L. divaricatum* (Michx.) Raf. (*Erigeron* Michx.) Frequent, especially in pastures.

Antennaria Gaertn. Lady's Tobacco, Pussy's Toes.

- 1465. *A. Parlinii* Fernald. Rare; Hardin Co. (L. H. Pammel).
- 1466. *A. plantaginifolia* (L.) Richards. Common in open woods.
- 1467. *A. neglecta* Greene. Our commonest species; prairies and woods.

Anaphalis DC.

- 1468. *A. margaritacea* (L.) B. & H. Pearly Everlasting. Frequent in dry soil.

Gnaphalium L.

- 1469. *G. obtusifolium* L. (*G. polycephalum* Michx.) Cudweed. Common. The plant has a fragrant odor.

1470. *G. purpureum* L. Purplish Cudweed. Rare in dry soil; Lee Co. (Jess L. Fults).

Inula L.

- *1471. *I. Helenium* L. Elecampane. Rare as an escape. (Europe).

Polymnia L.

1472. *P. canadensis* L. Leaf-cup. Frequent.

Silphium L.

1473. *S. laciniatum* L. Rosin-weed, Compass Plant. Common.
1474. *S. integrifolium* Michx. Frequent in the southeastern part.
1475. *S. perfoliatum* L. Indian Cup Plant. Common, especially in low, open woods.

Parthenium L.

1476. *P. integrifolium* L. Frequent.

Iva L.

1477. *I. xanthifolia* Nutt. (*Cyclachaena* Fresen.) Cattle-weed. Frequent in rich soil and waste places; perhaps an immigrant from the northwest.

Ambrosia (Tourn.) L. Ragweed.

1478. *A. trifida* L. Great Rag-weed, King's-head. Very common; fields and roadsides.
1479. *A. elatior* L. (*A. artemisiifolia* L.) Small Ragweed. Very common; roadsides and pastures.
1480. *A. media* Rydb. Rare; Monona Co. (L. H. Pammel).
1481. *A. coronopifolia* T. & G. (*A. psilostachya* Gray, not DC.) A perennial species with a running rootstock. Frequent; prairies and open woods.

Xanthium (Tourn.) L. Cocklebur.

1482. *X. americanum* Walt. (*X. canadense* Mill.) Infrequent.
1483. *X. glanduliferum* Greene. Rare; Emmet Co. (B. O. Wolden).
1484. *X. commune* Britton. Frequent.
1485. *X. echinatum* Murr. Our commonest species.
*1486. *X. spinosum* L. Rare; R.R. right-of-way. Story Co. (E. S. Smith) (Tropical America).

Heliopsis Pers. Ox-eye.

1487. *H. helianthoides* (L.) Sweet. Infrequent in the eastern part.
1488. *H. scabra* Dunal. Very common.

Eclipta L.

1489. *E. alba* (L.) Hassk. Infrequent in the southern part; perhaps introduced in Iowa.

Rudbeckia L. Cone-flower.

1490. *R. triloba* L. Common in woods.
1491. *R. subtomentosa* Pursh. Infrequent.
1492. *R. hirta* L. Black-eyed Susan. Very common.
1493. *R. laciniata* L. Common in woods; the original of the Golden-glow, a cultivated form.

Echinacea Moench. (*Brauneria* Neck.) Purple Cone-flower.

1494. *E. purpurea* (L.) Moench. (*Brauneria* Britton). Rare; Appanoose Co. (T. J. Fitzpatrick).
1495. *E. angustifolia* DC. (*Brauneria* Heller) Frequent.
1496. *E. pallida* (Nutt.) Britton. Common.

Ratibida Raf.

- 1497. *R. pinnata* (Vent.) Barnhart. (*Lepachys* T. & G.) Very common.
- 1498. *R. columnifera* (Nutt.) Wooton & Standley. (*R. columnaris* D. Don; *Lepachys* T. & G.) Infrequent near our western border.

Helianthus L. Sunflower.

- *1499. *H. annuus* L. Common in cultivation and as an escape. (Western Plains).
- *1500. *H. petiolaris* Nutt. Prairie Sunflower. Infrequent in waste places; a native of the western plains.
- 1501. *H. scaberrimus* Ell. (*H. rigidus* Desf.) Very common.
- 1502. *H. occidentalis* Riddell. Infrequent.
- 1503. *H. grosseserratus* Martens. Common, and a bad weed in cultivated fields.
- 1504. *H. laetiflorus* Pers. Infrequent, central and eastern.
- 1505. *H. Maximiliani* Schrad. Common in the northwestern part.
- 1506. *H. divaricatus* L. Infrequent.
- 1507. *H. leptocaulis* (S. Wats.) Blake. Rare; Des Moines Co., Sept. 1930. (L. H. Pammel). Determined by Miss M. Rae Johns.
- 1508. *H. strumosus* L. Common especially in or near timber; a variable species.
- 1509. *H. tuberosus* L. Wild Artichoke. A common tuberous rooted species, often cultivated under the meaningless name of Jerusalem Artichoke.
- 1510. *H. tuberosus subcanescens* Gray. Infrequent. Our wild sunflowers form a difficult group from a taxonomic standpoint, and much thorough field work is needed.

Actinomeris Nutt.

- 1511. *A. alternifolia* (L.) DC. Frequent in open woods.

Coreopsis L.

- *1512. *C. tinctoria* Nutt. A common garden annual, and frequently escaped; native farther west.
- 1513. *C. palmata* Nutt. Very common.
- 1514. *C. tripteris* L. Tall Coreopsis. Frequent in or near woods.

Bidens L. Bur Marigold.

- 1515. *B. frondosa* L. Common Stick-tight; a disagreeable weed.
- 1516. *B. vulgata* Greene. Common and resembling the preceding, but having larger heads, the mature akenes, brown.
- *1517. *B. bipinnata* L. Spanish Needles. Rare; Lee Co. Probably introduced from farther east. (Jess L. Fufts).
- 1518. *B. connata* Muhl. Common in swampy places.
- 1519. *B. comosa* (Gray) Wiegand. Infrequent in low places.
- 1520. *B. cernua* L. (*B. filamentosa* Rydb. and *B. gracilentia* Greene; the latter a depauperate form caused by very dry weather). Common in swamps, the heads nodding.
- 1521. *B. trichosperma* (Michx.) Britton. Rare in the northeast.
- 1522. *B. aristosa* (Michx.) Britton. Infrequent.
- 1523. *B. involucrata* (Nutt.) Britton. Common in the southern part and spreading northward; a handsome species.
- 1524. *B. Beckii* Torr. (*Megalodonta* Greene) Water Marigold. Rare in shallow water near Lakeside Laboratory, West Okoboji Lake, Dickinson Co. (R. B. Wylie)

Madia Molina.

- *1525. *M. glomerata* Hook. Rare; introduced from the western plains. Mahaska Co. (L. H. Pammel) Emmet Co. (B. O. Wolden).

Galinsoga Ruiz & Pavon.

- *1526. *G. parviflora hispida* DC. Becoming frequent in waste places. (Tropical Am.)

Helenium L.

1527. *H. autumnale* L. Sneeze-weed. Common. Includes several recent segregates.

Dyssodia Cav.

1528. *D. papposa* (Vent.) Hitch. Frequent in sterile ground, in or near timber.

Achillea (Vaill.) L. Yarrow, Milfoil.

- *1529. *A. Millefolium* L. Frequent. (Europe).
1530. *A. lanulosa* Nutt. Woolly Yarrow. Common; perhaps introduced from farther west.

Anthemis (Mich.) L.

- *1531. *A. Cotula* L. May Weed, Dog Fennel. Very common near dwellings. (Europe).
*1532. *A. tinctoria* L. Yellow Chamomile. A rare escape, Clayton Co. (Europe).

Matricaria (Tourn.) L.

- *1533. *M. suaveolens* (Pursh) Buchenau. Pine-apple Weed, the bruised foliage having a strong pineapple odor. An infrequent doorway weed. (Pacific Coast States).

Chrysanthemum (Tourn.) L. Ox-eye Daisy.

- *1534. *C. Leucanthemum* L. The typical form rare; Emmet Co. (B. O. Wolden) (Europe).
*1535. *C. Leucanthemum pinnatifidum* Le Coq. & Lamotte. Common as a weed. (Europe).

Tanacetum L.

- *1536. *T. vulgare* L. Tansy. A frequent escape. (Europe).

Artemisia L. Wormwood.

1537. *A. caudata* Michx. Common on dry knolls.
1538. *A. cernua* Nutt. Frequent. Prairies and open woods.
1539. *A. serrata* Nutt. Infrequent in or near timber.
1540. *A. gnaphalodes* Nutt. Very common.
1541. *A. pabularis* (Aven Nelson) Rydb. Rare in dry ground; Emmet Co. (B. O. Wolden).
1542. *A. Purshiana* Besser. Rare; Emmet Co. (B. O. Wolden)
1543. *A. biennis* Willd. Biennial Wormwood. Common and weedy.
*1544. *A. annua* L. Sweet-scented Wormwood. A garden escape. (Europe).
*1545. *A. Absinthium* L. Wormwood. Frequent as an escape. (Europe).
*1546. *A. Abrotanum* L. Southern-wood or Old Man. Rare as an escape. (Europe).

Erechtites Raf.

1547. *E. hieracifolia* (L.) Raf. Fireweed. Frequent.

Cacalia L.

1548. *C. suaveolens* L. (*Synosma* Raf.) Infrequent in open woods.

1549. *C. reniformis* Muhl. (*Mesadenia* Raf.) Infrequent in damp, rich woods.
 1550. *C. atriplicifolia* L. (*Mesadenia* Raf.) Pale Indian Plantain. Infrequent in rich woods.
 1551. *C. tuberosa* Nutt. (*Mesadenia* Britton) Common on low prairies.

Senecio (Tourn.) L.

1552. *S. palustris* (L.) Hook. Occasionally appears in marshy places in the northern part.
 1553. *S. Balsamitae* Muhl. (*S. aureus* var. T. & G.) Ragwort or Squaw-weed. Frequent northeast.
 1554. *S. plattensis* Nutt. Common Ragwort. Frequent on prairies.
 1555. *S. integerrimus* Nutt. Infrequent.

Arctium L. Burdock.

- *1556. *A. minus* Bernh. Common. The larger headed species, *A. Lappa* L. Probably occurs in the state. (Europe).

Echinops L.

- *1557. *E. sphaerocephalus* L. Globe Thistle. Cultivated, and rarely escaped. (Europe).

Carduus (Tourn.) L. Plumeless Thistle.

- *1558. *C. nutans* L. Musk Thistle. Infrequent. (Europe).
 *1559. *C. acanthoides* L. Infrequent in waste places. (Europe).

Cirsium (Tourn.) Hill. Thistle.

- *1560. *C. lanceolatum* (L.) Hill. Bull Thistle. Common, especially in pastures. (Europe).
 1561. *C. Flodmanii* (Rydb.) Arthur. (*C. canescens* Pammel not Nutt.) Woolly Thistle. Frequent.
 1562. *C. discolor* (Muhl.) Spreng. Field Thistle. Common.
 1563. *C. altissimum* (L.) Spreng. Frequent in the eastern part.
 1564. *C. iowense* (Pammel) Fernald. Common, especially on prairies. The var. *Crattyi* Pammel is only an ecological form with the leaves mostly entire.
 1565. *C. muticum* Michx. Infrequent in marshes.
 1566. *C. megacephalum* Gray. Rare in the northwestern part.
 1567. *C. undulatum* (Nutt.) Spreng. Rare in the northwestern section.
 1568. *C. Hillii* (Canby) Fernald. Frequent in dry soil.
 *1569. *C. arvense* (L.) Scop. Canada Thistle. A common, unlawful weed. Forms with the early leaves entire also occur. (Europe).

Onopordum (Vaill.) L.

- *1570. *O. Acanthium* L. Cotton or Scotch Thistle. Rare as an escape Fayette Co. (Europe).

Centaurea L. Star Thistle.

- *1571. *C. solstitialis* L. Barnaby's Thistle. Becoming frequent as a weed. (Europe).
 *1572. *C. Jacea* L. Infrequent. (Europe).
 *1573. *C. Cyanus* L. Bachelor's Button. A frequent escape from gardens. (Europe).
 1574. *C. diffusa* Lam. A recent introduction, but thoroughly established in Sioux Co. (A. L. Bakke). Native of S. E. Europe.

- *1575. *C. repens* L. Russian Knapweed. A deep-rooted perennial recently introduced in Sioux Co. (A. L. Bakke) It should be classed with our unlawful weeds. (Europe).
- *1576. *C. maculosa* Lam. Infrequent. (Europe).
- *1577. *C. nigrescens* Willd. Rare; Calhoun Co. (W. R. Hartley) Adv. from Europe.

Carthamus (Tourn.) L.

- *1578. *C. tinctorius* L. Safflower. Rare as an escape from cultivation; Sac Co. (Malcolm Rogers) (Europe).

Cichorium (Tourn.) L.

- *1579. *C. Intybus* L. Chicory. Frequent as an escape. (Europe).

Krigia Schreb.

- 1580. *K. amplexicaulis* Nutt. (*Adopogon virginicum* Kuntze, *Cynthia virginica* D. Don.) Dwarf Dandelion. Infrequent.

Picris L.

- *1581. *P. echinoides* L. Ox-tongue. Rare; Clarke Co. (E. E. Agans) Introduced from Europe.

Tragopogon (Tourn.) L.

- *1582. *T. pratensis* L. Goat's Beard. Frequent along roadsides. (Europe).

Taraxacum (Haller) Ludwig.

- *1583. *T. erythrospermum* Andr. Red-seeded Dandelion. Frequent. (Europe).
- *1584. *T. officinale* Weber. One of our commonest weeds in lawns and waste places. (Europe).

Sonchus (Tourn.) L. Sow Thistle.

- *1585. *S. arvensis* L. Perennial Sow Thistle. Becoming frequent and like the following very difficult to eradicate. (Europe).
- *1586. *S. uliginosus* Bieb. A perennial species, generally confused with the preceding, and more common. (Europe).
- *1587. *S. oleraceus* L. Common Sow Thistle, Annual or biennial; common in pastures and waste places. (Europe).
- *1588. *S. asper* (L.) Hill. Spiny-leaved Sow Thistle; less common than the preceding. (Europe).

Lactuca (Tourn.) L. Lettuce.

- *1589. *L. scariola* L. Prickly Lettuce. Common in cultivated and waste ground. (Europe).
- *1590. *L. virosa* L. (*L. Scariola integrata* of Manuals). Common; only the lower leaves lobed. (Europe).
- 1591. *L. canadensis* L. Common and variable.
- 1592. *L. sagittifolia* Ell. Frequent; the sagittate leaves clasping at the base.
- 1593. *L. ludoviciana* (Nutt.) DC. Frequent on prairies. Flowers usually purple with us.
- 1594. *L. pulchella* (Pursh) DC. Frequent.
- 1595. *L. villosa* Jacq. Frequent in the eastern part.
- 1596. *L. spicata* (Lam.) Hitch. Most frequent in the eastern part.

Lygodesmia D. Don.

- 1597. *L. juncea* (Pursh) D. Don. Frequent on morainic hills in the northwestern part.

Agoseris Raf.

1598. *A. cuspidata* (Pursh) Steud. (*Troximon* Pursh; *Nothocalais* Greene) Frequent northwest on morainic hills.

Crepis L. Hawk's Beard.

- *1599. *C. capillaris* (L.) Wallr. Rare in waste places; Poweshiek and Marshall counties. (Europe).
 *1600. *C. tectorum* L. Rare; Marshall Co. (Harley Walker) Adv. from Europe.

Nabalus Cass. Rattlesnake-root.

1601. *N. racemosus* (Michx.) DC. (*Prenanthes* Michx.) Frequent; banks of streams and on prairies.
 1602. *N. asper* (Michx.) T. & G. (*Prenanthes* Michx.) Frequent.
 1603. *N. albus* (L.) Hook. (*Prenanthes* L.) Common in rich woods.

Hieracium (Tourn.) L. Hawkweed.

- *1604. *H. florentinum* All. King Devil. Rare; Butler Co. (L. Crowley); a bad perennial weed. (Europe).
 *1605. *H. virosum* Pall. Determined at the Gray Herbarium. Rare, and not before reported from America. Floyd Co. (Dr. Ada Hayden) Howard Co. (Kathryn Shields). Introduced from s.e. Europe.
 1606. *H. scabrum* Michx. Infrequent.
 1607. *H. longipilum* Torr. Frequent in open woods and on prairies.
 1608. *H. canadense* Michx. Common, especially in open woods.

SUMMARY

Families represented	124
Genera	583
Native species and varieties	
Ferns and Fern Allies	46
Gymnosperms	6
Angiosperms	1263
Naturalized, escaped and adventive	293
Total number listed	1608

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A STUDY OF THE STARCH-DIGESTING AND THE SUGAR-FORMING ENZYMES OF WHEAT¹

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Investigators in a study of amylases have indicated the occurrence of an amylolytic enzyme with power to liquefy starch or to change starch to dextrins; and of a saccharogenic enzyme with power to change starch or dextrins to sugar. It is of interest to compare the methods used by these investigators, and the results upon which they base the theory that amylase consists of two enzymes, an amylolytic and a saccharogenic. Grützner (4) reported that by heating salivary amylase to 80°C., the saccharogenic activity is inhibited, and the amylolytic power is retained. Bourquelot (1), also, used heat at 68°C. to destroy the saccharogenic diastase, while the liquefying power is not changed. Teichek (15) determined the ratio of amylolytic to saccharogenic enzyme during various stages of malting and, since the ratio is not the same at these different periods of germination, he explained his observations as favoring the two-enzyme theory.

Sherman and Schlesinger (14) studied the properties of amylase from several sources; with pancreatic amylase preparations, they determined that the ratio of amylolytic to saccharogenic activity is 2 to 1. This same ratio of amylolytic to saccharogenic activity is noted in the power of the active enzyme precipitates obtained in the various procedures of the purification of pancreatic amylase. The authors consider that two enzymes with widely different properties would not pass through all the processes of purification and remain in the same quantitative ratio to each other.

Chrzaszcz (3) stated that the liquefying activity is lacking in resting seeds, and is developed upon germination. The saccharogenic activity is present in normal quantity in both resting and germinated grains. Ling and Nanji (5) point to a similar conclusion in stating that the enzyme of unmalted barley hydrolyzes amylose and not amylopectin; the enzyme which hydrolyzes amylopectin is developed on germination.

Van Klinkenberg (16) prepared a solid α -amylase of malt by precipitation with alcohol. This was redissolved and heated at 70°C. to destroy any β -amylase, then reprecipitated. β -amylase was prepared by extracting ground barley, first with fifty per cent alcohol and subsequent filtration, and then extraction with eighty per cent alcohol.

Ohlsson (8,9,10) supports the theory that amylase consists of a saccharogenic and an amylolytic enzyme. His experiments consist of a systematic study of the destruction of the enzyme by heat; at varying acidities, he studied the influence of temperatures ranging from 38° to 80°C. upon each activity of the enzyme. This work indicates that the saccharogenic enzyme is more easily destroyed by heat than the amylolytic. Ohlsson studied also the influence of hydrogen ion concentration upon each activity;

¹ From a thesis submitted to the Graduate Faculty of Iowa State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

the results indicate that the amylolastic enzyme is more readily affected by hydrogen ion concentration than the saccharogenic. From these data, Ohlsson suggests a method for the preparation of an amylolastic extract with little saccharogenic power, and a method for the preparation of a saccharogenic extract with little amylolastic power.

Since the amylase of malt and of wheat are similar in many properties, it was thought that the methods suggested by Ohlsson might be used for the preparation of the amylolastic and the saccharogenic enzymes of wheat. Working at temperatures near 0°C., Ohlsson brought the malt extract to a pH of 3.3 by the addition of hydrochloric acid, and then adjusted the solution to pH 6 by the addition of disodium hydrogen phosphate, and so obtained an extract in which a high percentage of the amylolastic activity was destroyed. The saccharogenic activity of this extract compared favorably with that of untreated extract. Thus, it should be possible to treat wheat extract in the same way, and from this extract, fractional precipitation with alcohol should yield a solid enzyme preparation with power to digest starch to sugars, and with little amylolastic activity. Ohlsson showed also, that the saccharogenic property of a malt extract can be almost entirely destroyed by heating for fifteen minutes at a temperature of 70°C., and that this treatment affects but slightly, the amylolastic property. Treating wheat extract in the same manner, it should be possible to prepare by fractional precipitation with alcohol a solid enzyme with power to digest starch to dextrans but with little sugar-forming power.

The object of this investigation was to prepare and study two solid enzymes, one showing strong amylolastic properties (and little saccharogenic), and the other showing strong saccharogenic properties (and little amylolastic).

EXPERIMENTAL WORK

1. *Preparation of the amylolastic enzyme.* Germinated wheat (ground) was extracted with twice its weight of cold distilled water. The extract was heated at 70°C. for fifteen minutes; during this time the solution was gently shaken. In the process of heating, a white precipitate appears. (This precipitate was found to have no enzyme activity.) At the end of the heating period, the extract was quickly cooled, and filtered. The material was placed in collodion sacs and dialyzed for 36 hours. The dialysate yielded active enzyme material by precipitation with absolute alcohol. The first precipitate was obtained when alcohol was added so that the dialysate was 35 per cent (the alcohol 65 per cent) of the final volume. A second precipitate was obtained by making the dialysate 15 per cent (the alcohol 85 per cent) of the final volume. The precipitates were dried in a vacuum over sulfuric acid. Each of these precipitates has an amylolastic activity that compares favorably with the activity of the precipitates obtained from untreated extracts. The saccharogenic activity of these precipitates was low (See table 1).

2. *Preparation of the saccharogenic enzyme.* The wheat extract, prepared as previously described and kept at 6° to 8°C., was brought to pH 3.3 by the addition of dilute hydrochloric acid. The addition of the acid was regulated in such a way that the amount required was added during fifteen minutes. Immediately, the hydrogen ion concentration was adjusted to pH 6 by the addition of a saturated solution of disodium hydrogen phosphate. This treated extract was placed in collodion sacs and dialyzed

for 36 hours. Fractional precipitation with alcohol, as previously described, yielded active enzyme precipitates.

All preparations described yielded enzyme precipitates with a saccharogenic power as high or higher than those obtained from untreated extracts. The amylolytic power of these precipitates was comparatively low (See table 2). Some difficulty was experienced in obtaining preparations with consistent saccharogenic powers; consequently, the procedure described was modified by varying the rate of addition of acid and by treating the extract in an atmosphere of carbon dioxide and of nitrogen.

3. *The activity of the solid enzyme precipitates.* (a) Methods for determination of the activity. By the expression, *amylolytic power of an enzyme* (14), is meant the number of milligrams of one per cent starch dispersion digested by one milligram of enzyme in one-half hour at 40°C.; the digestion occurs in the presence of optimum salt and hydrogen ion concentration. The two solutions necessary are: (1) an enzyme dispersion (in ice-cold distilled water) of known concentration, and (2) a one per cent starch (Lintner's) dispersion containing the required amounts of sodium hydroxide and phosphates for optimum activity (see sec. 4). The method for testing the amylolytic activity is based on that of Wohlgemuth (17) and depends on determining the quantity of enzyme which changes all the starch in five cubic centimeters of the dispersion to compounds that do not give a blue color with iodine. The procedure for determining this is as follows: A certain (but varying) quantity of enzyme dispersion is measured into each of several test tubes; to each tube is added 5 cc. of the starch dispersion. The mixture is placed in the thermostat at 40°C. for one-half hour. At the end of this time, the tubes are removed, and one drop of a solution of iodine in potassium iodide is added to each tube. The digestion products are blue in color in the tubes in which there is an insufficient supply of enzyme to digest all the starch. Other tubes may be red to yellow in color because of the formation of dextrins. In the series, the end point tube contains that weight of enzyme that digests all the starch to products which give a red-violet color with iodine. The amylolytic power of the enzyme, then, is determined by dividing the weight of the 5 cc. of one per cent starch dispersion (5,000 mg.) by the number of milligrams of enzyme present in the end-point tube.

The *saccharogenic power* (14) is the number of milligrams of maltose formed by one milligram of enzyme acting upon starch at optimum salt and hydrogen ion concentration, in one-half hour digestion at 40°C. It is necessary to have, as previously stated, an enzyme dispersion, and a two per cent starch dispersion containing the required amounts of sodium hydroxide and phosphates for optimum activity (see sec. 4). In making this test, the enzyme is allowed to act upon 100 cc. of the two per cent starch dispersion for one-half hour at 40°C. At the end of this time, 50 cc. of mixed Fehling's solution are added to the digestion mixture, and the flask placed in a boiling water bath for fifteen minutes. The cuprous oxide formed is a measure of the reducing sugar (maltose) present in the digestion mixture. The number of milligrams of cuprous oxide is converted into milligrams of maltose by Defren's tables (Leach, Food Anal., p. 619). The saccharogenic power is obtained by dividing the milligrams of maltose by the milligrams of enzyme.

(b) Discussion of results. Amylolytic preparations were made simultaneously, from treated and untreated enzyme extracts. In table 1,

the amyloclastic and saccharogenic powers are given for four enzyme preparations, as compared with an average standard (untreated extract) preparation. The loss in amyloclastic and saccharogenic activity is based on a comparison of the powers of each preparation with that of the standard preparation from untreated extract.

TABLE 1. *The saccharogenic and amyloclastic activity of the amyloclastic enzyme preparations*

Preparation	Amyloclastic	Saccharogenic	Percentage loss amyloclastic	Percentage loss saccharogenic
(Treated) 1	67,000	11	19	94
(Treated) 2	48,000	14	42	93
(Treated) 3	67,000	35	19	83
(Treated) 4	56,000	31	33	85
Standard (Ave)	83,000	211	—	—

The results shown in table 1 indicate that the four preparations made from treated enzyme extract have an amyloclastic activity that compares favorably with that of an average untreated (standard) enzyme precipitate. The loss in amyloclastic activity varies, but is only a little over 20 per cent. These amyloclastic preparations have but little saccharogenic activity, as compared to that of the precipitate obtained from untreated extract. The loss in saccharogenic activity is 85 to 95 per cent. That is, from a treated wheat extract, a solid enzyme precipitate has been obtained which has a high amyloclastic power (compared to a standard) and a very low saccharogenic power.

In the preparation of the saccharogenic enzyme, the extract was treated as previously described. Preparations were made simultaneously from treated and from untreated extracts. Table 2 shows the amyloclastic and saccharogenic powers obtained with eight preparations, as compared with an average standard enzyme precipitate obtained from untreated extract.

TABLE 2. *The saccharogenic and amyloclastic activity of the saccharogenic enzyme preparations*

Preparation	Amyloclastic	Saccharogenic	Percentage loss amyloclastic	Percentage loss saccharogenic
1	4,000	468	95	00
2	2,000	116	97	46
3	2,000	109	97	49
4	4,000	267	95	00
5	5,000	377	94	00
7	3,000	245	96	00
9	11,000	264	87	00
11	11,000	255	87	00
Ave. stand.	83,000	211	—	—

The results shown in table 2 indicate that nearly all of the preparations made from treated extract have a higher saccharogenic activity than the average preparation from untreated extract. These precipitates have little amyloclastic power, when compared to standard enzyme; the loss in amyloclastic activity is from 87 to 97 per cent. That is, from the treated wheat extract, a solid enzyme precipitate has been obtained which has a high saccharogenic power and very little amyloclastic activity.

4. *Determination of the hydrogen ion concentration for optimum activity of wheat amylase.* Preliminary experiments on the optimum hydrogen ion concentration for the saccharogenic activity of wheat amylase have been reported (7). Similar experiments to determine the optimum hydrogen ion concentration for amylolytic activity have not been made for wheat amylase. Moreover, recent work by Sherman, Caldwell, and Adams (12) and Sherman, Caldwell, and Dale (13) indicate that hydrogen ion adjustments may be accurately made with a mixture of primary and secondary phosphates, keeping the phosphate concentration constant. This seemed a better method for the adjustment of hydrogen ion concentration than that used in any previous work; therefore, this method was used in the experiments described.

The experiments were planned so as to make a systematic series of determinations of amylolytic and saccharogenic activity through the adjustment of hydrogen ion concentration with mono and disodium phosphates, until the optimum activity was exceeded and a distinct deleterious effect of the hydrogen ion concentration was observed. The hydrogen ion concentrations were determined with a glass electrode of the Mac Innes type (6).

The amylolytic determinations for two sets of experiments are described; in one set, the determinations are made in the presence of 0.02 molar total phosphate, and in the other set, the determinations are made in the presence of 0.06 molar total phosphate. The starch dispersions were made by using a concentrated starch paste, to which was added sufficient

TABLE 3. *Optimum pH for amylolytic activity of standard wheat amylase, 0.02 M total phosphate*

Volume in cubic centimeters		pH	Amylolytic power
NaH ₂ PO ₄ (0.2M)	Na ₂ HPO ₄ (0.2M)		
10.00	0.00	3.71	12,000
9.80	0.20	4.10	21,000
9.70	0.30	4.47	33,000
9.68	0.32	4.60	33,000
9.66	0.34	4.68	42,000
9.64	0.36	4.78	42,000
9.62	0.38	4.85	42,000
9.60	0.40	4.89	42,000
9.50	0.50	5.17	42,000
9.40	0.60	5.35	42,000
9.30	0.70	5.46	42,000
9.20	0.80	5.58	42,000
9.10	0.90	5.67	42,000
9.00	1.00	5.88	42,000
8.00	2.00	6.24	42,000
7.80	2.20	6.21	42,000
7.60	2.40	6.26	42,000
7.40	2.60	6.30	42,000
7.20	2.80	6.34	33,000
7.00	3.00	6.46	33,000
6.00	4.00	6.60	33,000
5.00	5.00	6.77	33,000
4.00	6.00	6.93	28,000
3.00	7.00	7.06	28,000
2.00	8.00	7.23	24,000
1.00	9.00	7.46	19,000
0.00	10.00	7.79	14,000

primary and secondary sodium phosphates to give a final concentration of 0.02 or 0.06 molar total phosphate when this starch was diluted to one per cent. The total phosphate concentration was constant, but the relative amounts of the two phosphates were varied in order to obtain the desired hydrogen ion concentration. Tables 3 and 4 show the amylolytic power obtained by the action of a standard wheat enzyme upon starch dispersions of a range of hydrogen ion concentrations from pH 3.71 to 7.79, and in the presence of 0.02 molar total phosphate (table 3) and of 0.06 molar total phosphate (table 4).

The data included in tables 3 and 4 show that the hydrogen ion concentration for optimum amylolytic activity of wheat amylase is at pH 4.6 to 6.3. In this range of hydrogen ion concentration, no measurable differences in the amylolytic activity could be detected in solutions containing 0.02 and 0.06 molar total phosphate. This work determines accurately the hydrogen ion concentration for optimum amylolytic activity of wheat amylase, and indicates that phosphate has no activating effect upon the amylase in the concentrations used. This finding is consistent

TABLE 4. *Optimum pH for amylolytic activity of standard wheat amylase, 0.06 M total phosphate*

Volume in cubic centimeters		pH	Amylolytic power
NaH ₂ PO ₄ (0.2M)	Na ₂ HPO ₄ (0.2M)		
29.0	1.0	3.63	12,000
28.5	1.5	4.02	17,000
28.0	2.0	4.76	42,000
27.0	3.0	5.40	42,000
26.0	4.0	5.66	42,000
23.0	7.0	6.07	42,000
21.0	9.0	6.24	42,000
19.0	11.0	6.39	33,000
17.0	13.0	6.53	33,000
16.0	14.0	6.59	33,000
15.0	15.0	6.64	28,000
14.0	16.0	6.70	28,000
13.0	17.0	6.75	24,000

TABLE 5. *Optimum pH for saccharogenic activity of standard wheat amylase, 0.02 M. total phosphate*

Volume in cubic centimeters		pH	Mg. Cu ₂ O	Saccharogenic power
NaH ₂ PO ₄ (0.2M)	Na ₂ HPO ₄ (0.2M)			
10.0	0.0	3.64	163.2	221
9.9	0.1	3.80	170.6	231
9.8	0.2	3.99	171.3	232
9.7	0.3	4.23	188.5	256
9.6	0.4	4.71	207.7	282
9.5	0.5	4.98	212.8	290
9.4	0.6	5.21	216.8	295
9.3	0.7	5.38	210.4	286
9.2	0.8	5.42	206.6	281
9.1	0.9	5.58	203.2	276
9.0	1.0	5.64	203.1	276
8.8	1.2	5.81	201.8	274
8.6	1.4	5.92	200.5	272
8.4	1.6	6.06	197.0	268

with the results obtained on saccharogenic sets with pancreatic amylase (13).

The hydrogen ion concentration for optimum saccharogenic activity was redetermined by the method just described, using 0.02 molar total phosphate. Two per cent starch dispersions, of varying hydrogen ion concentration and constant phosphate concentration, were prepared. Table 5 shows the saccharogenic power obtained by the action of a standard wheat enzyme upon starch dispersions of a range of hydrogen ion concentrations from pH 3.64 to 6.06, and in the presence of 0.02 molar total phosphate.

A critical study of these results indicates that there is a more decided optimum hydrogen ion concentration (pH 4.9 to 5.3) for saccharogenic activity of wheat amylase than had been previously determined. The results show also, that it is impossible to assume that the range of hydrogen ion concentration is the same for optimum amyloclastic and saccharogenic activity of amylase. The results here obtained indicate a broad optimum at pH 4.6 to 6.3 for amyloclastic activity, and a narrow optimum at pH 4.9 to 5.3 for saccharogenic activity.

SUMMARY

1. An amyloclastic enzyme solution and a saccharogenic enzyme solution has been prepared from wheat extract by a method similar to that used by Ohlsson for malt extract.
2. A solid amyloclastic enzyme with low sugar-forming power has been prepared by heating wheat extract for fifteen minutes at 70°C. and fractionally precipitating with alcohol.
3. A solid saccharogenic enzyme has been prepared from wheat extract by bringing the hydrogen ion concentration to a pH of 3.3 and then adjusting it to a pH of 6.0, and finally precipitating with alcohol. The solid enzyme showed an unusually high sugar-forming power and low amyloclastic power.
4. The optimum hydrogen ion concentration for the amyloclastic activity of wheat amylase has been found to be at pH 4.6 to 6.3. The range of hydrogen ion concentration has been obtained by varying the relative amounts of sodium dihydrogen phosphate and disodium hydrogen phosphate, thus keeping the total phosphate concentration constant. The optimum hydrogen ion concentration for saccharogenic activity has been determined by the method just indicated and found to be at pH 4.9 to 5.3.

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THE MICROSCOPIC ANATOMY OF THE DIGESTIVE TRACT OF *GALLUS DOMESTICUS*^{1,2}

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A study of the microscopic anatomy of the entire digestive tract of *Gallus domesticus* at different ages has never been undertaken. The author has never seen a related work illustrated with photomicrographs. These features added to the large compilation of literature included herein should make this a valuable source of reference to the anatomist. It should be of added value to the English speaking anatomist since previous to this time the more complete works were written in the German language.

This study was also considered with the idea that it would help solve a problem which has long confronted the pathologist. Since he is concerned with the effects of disease on organs, it is hoped that this paper will give him a standard for comparison.

REVIEW OF LITERATURE

Literature concerning the microscopic anatomy of the digestive tract of the bird is quite voluminous, but with one exception [Zietschmann, (1911)] no author includes the entire tract with all its appendages and he has not limited his description to *Gallus domesticus* alone. In many cases the work covered chiefly gross anatomy with an occasional reference to the microscopic structure. Authors dealing with the gross anatomy alone included Huxley (1878), Wiedersheim (1907), Kingsley (1917), Johnston (1920), Kaupp (1921), Latimer and Osborn (1923), and Ellenberger and Baum (1926). Baum (1930) has made quite a complete study of the lymph system of the digestive tract of the chicken.

MOUTH

Boetticher (1928), Bradley (1915), Chauveau (1905), Grossman (1927), Kaupp (1918), Krause (1922), Owen (1866), Wiedersheim and Parker (1897), Schauder (1923), and Zietschmann (1911), spoke of the "horny" beak of the bird. Boetticher gave the time of beginning cornification as the sixteenth day of incubation. Krause alone gave a complete histological description of the beak. He divided it into four layers: bone, subcutis, cutis and epidermis. According to Owen "the beak consists of an upper mandible supported by the maxillary and premaxillary bones, and of a lower mandible formed by the lower jaw." Schauder, Bütschli (1924), and Rosenstadt (1912) described the "Eizahn," a protuberance found on the upper beak of newly hatched chicks.

All recent authors are agreed that birds lack teeth. Blanchard (1860) described vestigial teeth in certain birds (parrots). Marsh (1881-82) stated that no true teeth had yet been found, and Ihde (1912) that further research along that line would be fruitless.

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Marschall (1895), Shufeldt (1890), and Owen (1866) described the tongue muscles. Hollis (1901) gave particular attention to the skeleton of the tongue as associated with its function. Zietschmann (1911), and Bradley (1915) described the relation of the shape of the tongue to the conformation of the mouth roof. Owen (1866), Wiedersheim and Parker (1897), and Ward and Gallagher (1927) spoke of it as a prehensile organ. Bütschli (1924), Grossman (1927), Wiedersheim and Parker, Otte (1928), and Schauder (1923) described the tongue as pointed, especially horny at the apical end, and poor in muscle. Kaupp stated that the body of the tongue was made up of muscle and connective tissue. Schauder said the tongue corresponded to the form of the beak while Marschall found that "the tongue of the chicken does not correspond to the form of the beak but has approximately the form of a shoe sole and is soft." Kallius (1905) dealt with the embryology of the tongue of the sparrow.

According to Bradley (1915), Grossman (1927), Heidrich (1905), Kaupp (1918), Otte (1928), and Ward and Gallagher (1927), the roof of the mouth is the hard palate. Marshall (1895) on the contrary, stated that all birds lack a palate; Ward and Gallagher, and Heidrich that a soft palate was absent. Heidrich gave a detailed histological description of the different layers in the wall of the mouth.

PHARYNX

All authors agreed that there was no exact line of demarcation between the mouth cavity and the pharynx. Killian (1888) stated that "birds have no naso-pharyngeal cavity." Grossman (1927) set aside the transverse row of papillae on the root of the tongue as a "convenient" mark for separating the two cavities while Heidrich (1905) designated a row of papillae in the palate for the same purpose. Heidrich (1905), and Zietschmann (1911) stated that the mouth-pharyngeal cavity is covered by a "cutaneous mucous membrane." The latter added that a stratum corneum was found only on the roof of the mouth and on the caudal part of the dorsum of the tongue. Bradley (1915) mentioned a stratified epithelium lining the whole of the mouth and pharynx. Zietschmann did not find any muscularis mucosae in the mouth-pharyngeal cavity while Heidrich described it as beginning in the pharynx. Gadow (1891b) said that the pharynx was thin walled and Heidrich that it had no muscle while Thomson (1923) designed the pharynx as a muscular region at the back of the mouth. Otte (1928) stated that instead of a soft palate a strong musculature was present. According to Schauder (1923) there were no voluntary muscles in the mouth except in the tongue.

It is the common belief that the sense of taste is not enjoyed by the chicken but taste cells were found on the tongue and hard palate by Schauder (1923), in the beak and tongue by Krause (1922), and in the mucous membrane between the sides of the lower beak by Otte (1928). Botezat (1906) found that chickens have a sense of taste resembling that of mammals. He found taste organs in the throat region.

Heidrich (1905) and Zietschmann (1911) described macroscopic papillae which have a matrix belonging to the tunica propria. Zietschmann stated that in the anterior part of the roof of the mouth there was little lymphoid tissue but that it increased posteriorly until the maximum was reached in the region of the opening of the Eustachian tubes. Killian (1888) has designated a certain area of adenoid tissue as "throat tonsil"

especially in the region of the openings of the Eustachian tubes between the epithelium and the throat glands.

SALIVARY GLANDS

All authors who discussed the mouth parts at all mentioned salivary glands. Heidrich (1905), Holting (1912), and Zietschmann (1911) wrote the most complete works on the subject. The first two agreed with two exceptions. Heidrich found basket cells while Holting did not and Heidrich perceived some change in the gland according to the physiologic state while Holting failed to do so. Zietschmann differed from Heidrich on three points. Heidrich gave fifty as the number of openings of the lateral palatine glands and ten to fifteen openings for the anterior submaxillary gland, while Zietschmann gave "approximately one hundred" for the former, and forty for the latter. Heidrich found muscle fibers in the gland capsule and Zietschmann did not. Bradley (1915), in his work referred to Heidrich. Kovacs (1928) found the salivary glands to be uniformly constructed, in contrast to Heidrich, who described three different forms. Chauveau (1905), Marshall (1895), and Owen (1866) described them as being little developed. Other authors mentioned them briefly: Browne (1922-23), "true salivary glands are absent"; Thomson (1923) mentioned their role in lubrication; Schauder (1923) "numerous glands purely mucous"; and Grossman (1927) spoke of several glands being in the submucous tissue producing a mucous secretion which did not contain a digestive enzyme. Schauder gave the location of all the glands but did not include the histology of them. Kaupp (1918) gave the location of angular, sublingual and palatine glands. Otte (1928) spoke of the first two and in addition, the submaxillary and the spheno-pterygoideae salivary glands. Bütschli (1924) mentioned particularly diffuse glands of the tongue. Owen mentioned the following: "folliculi lingualis," "glandulae sublinguales," "glandulae submaxillares," "glandulae anguli oris," "folliculi preglottidei," "folliculi post-nasales," and "amygdalae." Cholodowsky (1892) described the glands of the lower mandible and the "glandula angular oris." Wieder-sheim and Parker (1897) compared the lingual and palatine glands of the bird to those of the reptiles. Zietschmann described elastic fibers in the connective tissue propria in which the glands lie, as well as in the gland capsule itself.

ESOPHAGUS

In general the esophagus has been described as a very elastic tube extending from the pharynx to the proventriculus and containing at its entrance into the thorax a dilatation called the crop. Kupfer (1908) used the term pharynx instead of esophagus to designate the part from the back of the mouth to the proventriculus. There are, however, two markedly contrasting views as to its structure. Barthels (1895), Batt (1925), Bradley (1915), Browne (1922-23), Heidrich (1905), Kaupp (1918), and Kovacs (1928) spoke of the outer layer of muscle as a longitudinal one, while Cazin (1888b), Gadow (1879), Marshall (1895), Newton (1893-97), Otte (1928), Owen (1866), and Zietschmann (1911) mentioned an outer transverse or circular muscle layer. Heidrich observed that smooth muscle began quite a distance anterior to the esophagus.

Kovacs (1928), Schauder (1923), and Zietschmann (1911) found an esophageal tonsil near the lower extremity of the esophagus.

Another difference in opinion was manifested as to the number of layers in the wall. Zietschmann (1911), Kupfer (1908), and Otte (1928), said three; Batt (1925), Grossman (1927), and Marschall (1895), four; and Newton (1893-97), five.

The esophagus was described quite briefly by the following authors: Browne (1922-23), Bütschli (1924), Grossman (1927), Wiedersheim and Parker (1897), Thomson (1923), and Ward and Gallagher (1927). Schreiner (1900) and Schauder (1923) mentioned chiefly the longitudinal folds of the mucous membrane, smooth muscle, a longitudinally arranged elastic tissue sheath besides the muscularis mucosae, and the mucous glands. Of particular note in Barthels' (1895) work were "border cells," a detachment of the marginal edge of the mucosa.

Klein (1871) and Rubeli (1890) dealt with the structure, and Schumaker (1926) with the structure and development of the mucous glands. Batt (1925) stated that the mucous glands were most numerous in the upper esophagus. Michalka (1924) found pavement epithelium in the glands of the esophagus.

CROP

It was agreed that the crop had the same general structure as the esophagus. Batt (1925), Browne (1922-23), Chauveau (1905), Kaupp (1918), Marschall (1895), Otte (1928), Schauder (1923), Ward and Gallagher (1927), and Wiedersheim and Parker (1897) merely stated that mucous glands were present. Barthels (1895) found no glands in the diverticulum of the crop. Schreiner (1900) found glands only on the "back side." According to Kupfer (1908) "the ventral surface of the crop and the side parts in the ventral surface are free from glands." Owen (1866) described "muciparous follicles" as being larger and more numerous than those of the esophagus. Gadow (1891b) found the crop to have a glandless lumen and Kovacs (1928) found glands in the dorsal wall. Browne (1922-23) found them most numerous near its openings. Schauder (1923) wrote that the middle part of the ventral crop wall had no glands. Klein (1871) and Bradley (1915) found them absent in the crop. Zietschmann quoted Barthels, Gadow, and Schreiner on the matter of mucous glands and said in addition that the crop lacked lymphoid tissue.

PROVENTRICULUS

Bischoff's (1838) work brings forcibly to one's mind the advancement made in histology in the last century. A quotation from his work follows: "The mucous membrane of the proventriculus which is present here and which is separated from the little sacs is in the form of small pyramidal pouches or villi in which I could not observe any epithelium but only a granular structure. . . . If one sections it with a fine pair of scissors and flattens it out. . . ."

In general the proventriculus was described as having a mucous membrane lined with simple columnar epithelium and containing in the tunica propria superficial tubular glands, a muscularis mucosae next, and between it and the lamina muscularis were the deep propria glands, [Bradley (1915), Zietschmann (1911)]. In contrast to this Batt (1925) described the deep propria glands as being between the muscularis mucosae and the epithelium. There were differences of opinion as to the structure of the glands. Batt, Bradley, Browne (1922-23), Grossman (1927), and Otte (1928) described

the deeper glands as tubular. According to Cazin (1887b and 1888a) the glands were "by no means tubular." Cazin (1887c) mentioned them as "culs-de-sac." Kovaes (1928) stated that the deep glands were sac-formed. Schreiner (1900) and Zietschmann (1911) found them to be multilobar. Wilizewski (1870) stated that the glands increased in size near the esophagus and decreased near the gizzard, while Marshall (1895) found the glands to decrease at both extremities. According to Gadow (1891b) glands were located in approximately fifty scattered rows. Newton (1893-97), Ward and Gallagher (1927), and Wiedersheim and Parker (1897) mentioned particularly the large amounts of glandular tissue in the proventriculus.

Kovaes (1928) stated that "the glands may be likened to fundus glands"; Browne (1922-23) that the proventriculus was "analogous to true stomach in mammals"; Bütschli (1924) compared the proventriculus to the cardiac portion of the mammalian stomach; Batt (1925) stated that there were no acid or peptic cells in the proventriculus but that they resembled the parietal cells of mammals; and Kaupp (1918) found them similar to the fundic glands of the horse.

The same differences of opinion as to the muscle layers existed here as in the esophagus. Cazin (1888b) admitted that a longitudinal layer might be seen on the outside of the external circular layer. He also found the muscularis mucosae to contain both a longitudinal and transverse layer. Zietschmann (1911) made this statement, "Of twenty-six investigated kinds of birds, only one, *Ictomes calidris*, lacked the longitudinal layer." In Oppel's (1896) work on the muscular layers of the proventriculus he described an outer longitudinal layer (often rudimentary) and an inner circular layer comprising the lamina muscularis, and an inner longitudinal layer and the muscle around the glands as making up the muscularis mucosae. Zietschmann stated that the outer longitudinal layer ended at the beginning of the gizzard.

Zietschmann (1911) described a lymphocytic infiltration of the propria which included many eosinophils. Hässe (1866) found only a trace of elastic elements visible. Laroche (1926-27) quoted Cazin. Swenander (1902) studied *Gallus domesticus* in comparison with other birds. Chauveau (1905), Owen (1866), and Schauder (1923) gave slight consideration to this organ. Schreiner's (1900) work included quite a detailed cytological study of the epithelium. At the point of the folds prismatic cells had a height of thirty microns. The basal part of the cell was small, granular, and contained an oval nucleus. Other vacuolated cells which took a deeper stain were described. He also found the cells to diminish in height as the fold widened.

According to some authors there is a so-called "intermediary piece" between the proventriculus and the gizzard. Batt (1925) said it was analogous to the mammalian pylorus. Schauder (1923), Zietschmann (1908), Kovaes (1928), Hässe (1866), and Cazin (1886b) stated that it was characterized by the lack of the deep propria glands of the proventriculus. Cazin (1886a) described the tubular glands as longer than the surface glands of the proventriculus. Cazin (1888b) stated that the exudate was more complex. Schauder and Zietschmann agreed that it lacked the hornified layers of the gizzard while Kovaes characterized it by its keratinized layer. Marshall (1895) found a sphincter of circular muscle fibers separating the proventriculus and gizzard, while Bradley (1915) designated the con-

striction only as a demarcation. Bütschli (1924) stated that a "clear intermediary piece is seldom inserted between the two sections."

VENTRICULUS

Most authors agreed on the general structure of the gizzard; its peculiar musculature, a thin submucosa and the mucous membrane with its glandular layer and its keratinized secretion forming an inner layer. Bradley (1915), Cornelius (1924), Gadow (1891b), Grossman (1927), Newton (1893-97), Otte (1928), Owen (1866), and Wiedersheim and Parker (1897) described the internal layer as hard, thick, yellowish, horny and keratinized. Zietschmann (1911) said it was falsely called a horny layer and Hedenuis (1892) described it as a keratinoid layer medial between keratin and albumin. Cazin (1887a) stated that it was not analogous to tegumentary coverings.

According to Bütschli (1924) this layer was strata-like and contained cast-off cells and Hässe (1866) mentioned parallel lines in it. Cazin (1886b) described a "secretion in the form of colonnades between which the recreation from the superficial epithelium is poured."

Browne (1922-23) spoke of a "horny epithelium" and stated that there were no true glands present. Otte (1928) said that the true mucous membrane lay under the inner membrane and that the glands therein resembled the stomach glands of mammals. Kovacs (1928) and Schauder (1923) compared them to pyloric glands of mammalian stomachs. Bütschli (1924), Hässe (1866), and Cazin (1886b) described the glands as tubular, Cazin (1886a) as long cylindrical culs-de-sac. Cornelius (1924), Hässe (1866), Kovacs (1928), and Zietschmann (1911) mentioned the cluster arrangement of the glands.

Cazin (1888b) stated that the cells were arranged obliquely to the axis of the tube. Pilliet (1886) stated that the gland cells were not cylindrical and Zietschmann (1911) that they were cubical to flat. Wiedersheim (1872) described secretion cells which appeared on the edge of the glands in profile. He also included a microscopic study of the secretion cone and secretion hook, the two latter being in contact with the cell itself. Zietschmann stated that the glands were shorter where the mucosa was thinner. He also found elastic tissue to be confined to an area beneath the gland region. Cazin (1885), in writing of the development of the cornified layer stated that on the sixteenth day of incubation the glands approached the adult form. Kaupp (1917) stated that walls of gizzards of granivorous birds were very thick while Magnan (1911a) contended that the muscle masses were reduced to a minimum. Batt (1925) mentioned a thin outer longitudinal muscle layer.

Both Garrod (1872), who wrote a paper on the mechanism of the gizzard, and Ashcraft (1930), in writing of the activities of the alimentary canal of the fowl, stated that in hunger the proventriculus and gizzard were vigorously and continually contracting, but did not discuss the minute structure of the gizzard. Bauer's (1901) work on the histology of the gizzard was chiefly done on the duck. Cazin (1887b) dealt with the embryonal development.

Marschall (1895) and Kaupp (1918) designated a valve and Otte (1928) a fold in the mucous membrane as separating the gizzard from the duodenum.

Marschall (1895) also stated that the wall of the gizzard became thin-

ner as it approached the duodenum. According to Zietschmann (1911) the border zone was rich in lymphocytes. Oppel (1896-1914) stated that this transitional zone corresponded to the pyloric region of mammals. Zietschmann called it the pyloric gland zone of the gizzard.

According to Oppel (1896-1914) one cannot speak of a pyloric sphincter between the ventriculus and the duodenum. He also stated that the circular muscle of the intestine could be considered as a continuation of the diminished muscles of the ventriculus. Zietschmann (1911) stated that the surface of the gland layer in this intermediary portion became uneven and the glands were farther apart and took on an aspect of villi. Oppel described the glands in this area as club-shaped and curved at their lower end—not a beginning of Lieberkühn's glands. Kovacs (1928) described an alteration of the glands and the presence of lymphoid infiltration possessing follicular character.

SMALL INTESTINE

Clara (1926a), Batt (1925) and Zietschmann (1911) agreed in general on the structure of the small intestine (duodenum included): the mucous membrane was lined with simple columnar epithelium interspersed with goblet cells; the villi were tongue shaped, longer and more numerous in the duodenum; a submucosa in which the blood vascular system was contained; and two layers of muscle—an inside muscularis mucosae, the middle circular and outer longitudinal layers of the lamina muscularis. Cloetta (1893) stated that there was no submucosa and that the blood and lymph vessels were in the tunica propria.

Two bile ducts and three pancreatic ducts opening into the duodenum were described for the chicken by all authors with the exception of Batt (1925) who described one bile duct and two pancreatic ducts. According to Gadow (1879) the entrance of the ducts into the intestine was marked by a small warty projection which contained a valve.

Newton (1893-97) described a villus as a structure containing a prolongation from the submucosa, a lacteal, arteries and veins and smooth muscles. Bujard's (1906) work indicated a change in the villi according to age. Clara (1927b) found the villi to present a picture of geometric regularity upon cross section.

Cloetta (1893) described the epithelial cells of the glands as being smaller than the epithelial cells of the villi. He also found goblet cells nearer the tip of the villus as the age of the birds increased. Zietschmann (1911) found that the epithelial cells contained a cuticular border. Greschik (1922) and Clara (1926b and 1927a) agreed that there were cells of Paneth while Cloetta doubted their presence. All authors with the exception of Kaupp were agreed that Brunner's glands were lacking. Kaupp (1918) made the following statement: "Openings of simple intestinal tubular glands the duodenal glands, or the glands formerly known as Brunner's glands, are located between the villi."

Otte (1928) described Peyer's patches in the bird intestine and Retterer and Lelievre (1910a) found areas having the appearance of Peyer's patches.

Zietschmann (1911) found that elastic fibers were not demonstrable in the gland layer and were comparatively few in other layers. Batt (1925) described a layer of white fibrous tissue between the outer longitudinal and the inner circular muscle. According to Batt there were valvulae con-

ventes present; also lymph nodules were fewer in the remainder of the small intestine than in the duodenum.

Browne (1922-23) made the statement that the intestine was uniform in caliber throughout while Cloetta (1893) and Otte (1928) found the duodenum to have a wider lumen.

Newton (1893-97), Thomson (1923), and Gadow (1891b) mentioned the ileo-cecal valve and Zietschmann (1911), an iliac sphincter. Wieder-sheim and Parker (1897), Marschall (1895), Ward and Gallagher (1927), and Grossman (1927) described the small intestine very briefly.

LARGE INTESTINE

The term large intestine was seldom used. Some used the term *colon* while others used the term *rectum* to include the portion from the caeca to the cloaca. Still others used the terms combined—*colon and rectum*. In this paper *rectum* will be used to refer to this portion.

CAECA

Zietschmann (1911), and Looper and Looper (1929) made the most complete studies of the caeca. The general structure corresponded to the small intestine. According to Eberth as reported by Bradley (1915), Kaupp (1918), Oppel (1897), and Zietschmann, there was an elevation in the caeca about two to four millimeters from their origin. Muthmann (1913) found so-called "caecal tonsils." Looper and Looper found that lymph nodules first appeared at about fourteen days in the tunica propria two millimeters from the origin of the caeca, and in the tunica propria and submucosa of the blind ends. Prior to this age the lymphoid tissue was scarce and diffuse. Berry (1900) found lymphoid tissue diffused throughout the mucosa. Bradley (1915), and Looper and Looper (1929) described many lymphoid nodules in the caeca. Batt (1925) stated that there were "few small lymphatic nodes" and Zietschmann (1911) that follicles seldom appear.

Looper and Looper (1929) found the muscularis mucosae to be absent in many places while Batt (1925) stated that it was well developed.

Looper and Looper found many eosinophils throughout the wall and Muthmann (1913) stated that cells with large granules were present in large quantities. Bittner (1924) and Otte (1928) divided the caeca into three parts: a neck with many villi; a middle portion with few villi; and the vesicular blind end which was thin walled and free from villi. Browne (1922-23) and Zietschmann (1911) stated that the villi were short or absent in the dilated portion. According to Batt (1925) the mucous membrane was thrown into folds which gave the appearance of villi.

Oppel (1896) referred to Eberth as finding ciliated epithelium in the folds and extending into the glands. Maumus (1902) attempted to verify this but failed. He concluded they were probably artifacts.

Zietschmann (1911) found goblet cells to be lacking in spaces where lymphoid tissue was numerous.

Other important facts brought out by Looper and Looper (1929) were: the submucosa was occupied by or obliterated by lymph nodules where the lymphoid tissue was present in the tunica propria; circular layers of muscle were displaced by lymphoid tissue in the blind end; reticular connective tissue fibers extended into the circular muscle layers and encircled the fibers; most important of all, the mucosa of the distal two thirds underwent

a degenerative change as fowls became older. The regression involved the atrophy of the epithelium and glands accompanied by the appearance of lymphoid tissue. This had, in turn, been replaced by sclerotic fibrous tissue in the blind ends of the caeca in a three-year-old specimen.

Ward and Gallagher (1927), Wiedersheim and Parker (1897), Marschall (1895), Otte (1928), Schauder (1923), and Grossman (1927) gave slight consideration to the caeca. Maumus and Launoy (1901), Mangold (1931), and Röseler (1929) dealt primarily with the physiology of these organs.

RECTUM

Regarding the structure of the rectum, little has been said beyond the fact that it was very similar to the small intestine. Owen (1866) stated that the villi of the rectum were coarser, shorter and less numerous than those of the small intestine. Greschik (1912) on the contrary found them to continue the same height to the anus. Zietschmann (1911) in describing the "cloacal end of the rectum" agreed with Owen (1866) and added that the villi "afterwards take on the greatest length of anywhere in the intestine." Grossman (1927) and Marschall (1895) described numerous villi with glands emptying between them. Greschik mentioned simple tubular glands and Zietschmann stated that the glands were longer than in the rest of the intestine. Clara (1926a), Greschik, and Zietschmann found lymphoid tissue in the rectum. According to Greschik, the submucosa was weakly developed and in many cases not apparent.

CLOACA

Bütschli (1924) found a sphincter marking the limitation of the rectum. Otte (1928) and Owen (1866) made the statement that the rectum terminated in a valvular circular orifice. Kaupp (1917) agreed with them by saying that there was a strong oblique fold of the mucous membrane where the large intestine emptied into the cloaca. According to Retterer (1885) and Jolly (1915) there was no demarcation between the two.

Ward and Gallagher (1927), Thomson (1923), Gadow (1891a), Bütschli (1924), Schauder (1923), and Bradley (1915) described three compartments in the cloaca. The most anterior was named coprodaeum, the middle one urodaeum, and the posterior one proctodaeum. Retterer (1885) spoke of a "rectal vestibule" in describing the anterior compartment. Owen (1866) stated that the rectum terminated in a rudimentary urinary bladder.

According to Gadow (1891a) the coprodaeum had the same mucous membrane as the rectum, and Zietschmann (1911) described a one-layered epithelium extending as far as the anal opening.

ANUS

Retterer (1885) described a sphincter of smooth muscle outside of which was a voluntary transverse cloacal muscle. Marschall (1895) described the anal opening as an oblique slit while Gadow (1891a) stated that it was a round opening.

LIVER

Few writers have given much attention to the microscopic anatomy of the bird liver. Krause (1922) studied the liver of the pigeon as repre-

sentative of the livers of birds and found it to be very similar to the mammalian liver. He found indications of changes in the cells according to the secretory condition. Batt (1926) also noted the similarity to the mammalian liver. Zietschmann (1911) stated that the liver of the bird had a smaller lobule design than the mammal. According to him central veins were lacking. Batt stated central veins and a portal system were present. Zietschmann found elastic fibers only in the vessel walls. Batt described a scant reticulum in the liver of the bird. Shore and Jones (1889) described the liver parenchyma as dense with obscure cell outlines. In young chickens the cell structure was clearly tubular with five rows of cells to the tubules. Shore and Jones further indicated that there was no distinction between interlobular and intralobular vessels. Shore (1890-91) found the liver cells of baby chicks to be excavated by spaces for oil droplets. He suggested a relationship between the color of the liver and the yolk. Doyle and Mathews (1928) stated that the color of the liver changed from the yellow of the baby chick liver to the red or maroon of the adult liver by the time a chick was a week to ten days old.

GALL BLADDER

The wall of the gall bladder as described by Zietschmann (1911) consisted of an adventitia with many blood vessels and some lymph follicles, an outer longitudinal and inner circular muscular layer, and a mucous membrane with many folds. The propria was filled with lymph cells. According to him, "the surface epithelium resembles that of the liver," and the surface was pouched and contained short crypts. Otte (1928) found many tubulose glands in the tunica propria.

The structure of the ducts was similar to that of the gall bladder. The ducts contained, according to Zietschmann (1911), a one layered cylindrical epithelium, while goblet cells and special glands were lacking.

PANCREAS

Zietschmann (1911) found the pancreas to differ little from that of the mammal. Pognat (1897) described three lobes each possessing a distinct excretory duct. Clara (1923-24) described in addition to the two lobes in the loop of the duodenum, a splenic lobe. He did not find a separate excretory duct for it. Clara found this splenic segment to be of a lobular structure while the other two were not. According to Pognat the pancreas was a ramifying and reticular tubulo-acinar gland. Krause (1922) and Clara described more islets of Langerhans in the dorsal lobe than in the ventral. According to Zietschmann the islets of Langerhans showed nothing special in the bird while Clara described a "pseudo-islet" similar in structure, but different in staining affinity. According to Batt (1926) they were smaller than the islets of the mammalian pancreas but similar in structure. Böhm (1904) did not find a pronounced accumulation of Langerhans cells. He did not find the islets to be set off from the surrounding pancreas while Batt described a delicate fibrous capsule surrounding them. Zietschmann and Krause found few centro-acinar cells while Pognat stated that there were no centro-acinar cells. According to Pognat the pancreatic cell was small. Batt found basket cells forming a reticulum about the acinar cells. Zietschmann described scant elastic tissue between the gland tubes.

Zietschmann (1911) stated that the pancreatic ducts were similar in

structure to the bile ducts. Batt (1926) mentioned a simple columnar epithelium lining the main duct and involuntary muscle fibers in its walls.

BURSA CLOACAE

The bursa cloacae (bursa of Fabricius) has been the source of much speculation in regard to its function. According to Retterer and Lelievre (1913b) it had previously been given the names, egg reservoir, third caecum, anal gland, anal pouch, urinary vessel, bladder, genital apparatus, seminal vesicle, prostate, Cowper's gland, and one author even described it as a pouch characteristic of the female which received the sperm of the male. Microscopically this organ has in general been described as having a serosa, a muscular tunic of smooth fibers, and a mucous membrane of longitudinal folds made up of a mass of lymphoid and epithelial tissue. Retterer (1885) described a thick serosa, Osawa (1911) a thin serosa, and Jolly (1915) stated that the capsule was made up of a thin connective tissue layer and a thin smooth muscle layer. Gadow (1891a) also mentioned the smooth muscle. Retterer (1885) found the exterior muscle layer longitudinal and the internal transverse, while Osawa found the opposite arrangement. Retterer (1885) stated that there might be as many as forty to fifty follicles in a single fold. Jolly (1915) stated that there were twelve to fourteen folds in the chicken.

There were two opposing views on the structure of the follicle: one that there was a connective tissue network and blood vessels in the medullary portion [Retterer (1885), Stieda (1880), and Osawa (1911)]; the other that connective tissue and blood vessels did not penetrate the medullary part [Schumacher (1903), and Wenekebach (1889 and 1896)]. Retterer and Lelievre (1910a) described an abundance of elastic fibers in the "inter-follicular walls."

There were some differences of opinion regarding the epithelium. Jolly (1915) mentioned only cylindrical epithelium; Schumacher stated that the epithelium varied from cuboidal and tall columnar to pseudo-stratified columnar; according to Osawa (1911) the epithelium was stratified with the surface layer cylindrical. Wenekebach (1889) did not find any goblet cells. Gadow (1891a) found few goblet cells in the epithelium of the bursa cloacae.

Forbes (1877) could not find a valve or flap over the opening to the bursa cloaca. Retterer (1885) stated that the posterior face of the uroanal fold overhung the opening into the bursa in *Uria troile*.

Jolly (1910, 1911a and 1913a), Retterer (1893), and Retterer and Lelievre (1913a) concerned themselves chiefly with the development of the bursa cloacae. Retterer and Lelievre (1910b and 1913b) stated that the medullary portion was of epithelial origin. According to Retterer (1885) its size at its maximum development was probably two and five tenths centimeters in length, two centimeters in width and one and five tenths centimeters in thickness. Jolly (1913b) stated that the beginning of involution coincided exactly with the appearance of sexual maturity while Riddle (1928) found involution usually complete coincident with sexual maturity. According to Schauder (1923), Otte (1928), Bradley (1915), and Kaupp (1918) its maximum growth was reached between four and five months. Bittner (1924) compared the bursa cloacae to an acorn at five months and a hemp seed or a pea at one year. Others such as Ward and Gallagher (1927), Thomson (1923), Marshall (1895), Gadow (1891a),

and Wiedersheim and Parker (1897) discussed its retrogression briefly. Jolly (1911c and 1915) wrote more in detail on the subject. Boyden (1922) referred to Jolly (1911b) on the matter of its physiological function, who ascribed to it a hematopoietique function. Jolly (1911b) and Jolly and Levin (1911) found that fasting had a rapidly degenerative effect on the bursa cloacae.

DIVERTICULUM

The remnant of the yolk stalk was described as an appendage to the small intestine. Otte (1928), Owen (1866), and Retterer and Lelievre (1910c) observed this diverticulum in many birds. Zietschmann (1911) stated that in all birds, except the goose, it disappeared completely after birth. Muthmann (1913) found it to remain during the entire life of the bird. Latimer (1924) found it to be constantly present in the chicken. Maumus (1902) stated that the cells lining the so-called third caecum were a continuation of the intestinal canal. Much lymphoid tissue was present. As age increased macrophages became numerous, the villi diminished, and the longitudinal muscle fibers near the blind end seemed to disappear little by little. Maumus made the statement that the disappearance of the muscle varied with the activity of the macrophages. Generally it began to disappear about the third month and had completely disappeared in the greater part of Gallinacea two months later.

The disappearance of the yolk sac itself was considered by Schilling and Bleecker (1928) to be almost complete by the fourteenth day. Schauder (1923) stated that it underwent involution at the sixteenth day in the chick. Latimer (1924) found the yolk sac, with one exception, up to and including the thirty-eighth day, and thereafter frequently up to the two hundred thirty-seventh day.

GROSS ANATOMY

Prehensile and masticatory organs are limited to an upper and lower beak. (Pl. I-1).

The chicken possesses a hard palate which is separated from the pharynx by a row of papillae. (Pl. II-5). The nares (Pl. II-4) open through a longitudinal slit into the middle of the hard palate and the dorsal midportion of the pharynx.

The tongue (Pl. II-12) is attached to the caudal part of the floor of the mouth and conforms to the shape of the beak. On its posterior part is a row of papillae. (Pl. II-13). The tongue muscles include lingual and hyoid muscles [Owen (1866) and Shufeldt (1890)].

The pharynx (Pl. II-6) is a poorly defined area in the back of the mouth into which open the Eustachian tubes (Pl. II-8), esophagus (Pl. II-11), larynx, mouth, and the nasal openings as described above. The aditus laryngis (Pl. II-16) presents no epiglottis.

The esophagus (Pl. I-5) is a long dilatable tube leading from the pharynx to the proventriculus with an outpouching, the crop (Pl. I-6), at the entrance into the thoracic cavity.

The proventriculus (Pl. I-7) appears as a dilatation of the esophagus at its posterior extremity but upon palpation is found to be thicker walled and spongy. It opens after a narrow constriction at its posterior extremity into the muscular stomach, gizzard or ventriculus.

The ventriculus (Pl. I-8) is oval, flattened laterally and particularly

prominent because of its musculature. This muscular mass is comprised of two pairs of muscles: the two thin muscoli intermedii (Pl. I-10), one of which arises near the proventriculus and the other at the posterior end, are between the thick muscoli laterales, (Pl. I-9) which are part of the wall of the lumen. Both pairs have their insertion on a tendinous aponeurosis (Pl. I-11) on the lateral side of the ventriculus.

The duodenum (Pl. I-12), the fore part of the small intestine, forms a loop in which the main part of the pancreas lies. Three pancreatic and two bile ducts enter at a point approximating the junction of the duodenum with the remainder of the small intestine.

The jejunum and ileum (Pl. I-16) between which there is no line of differentiation are arranged in coils supported by the mesentery.

At a point about midway in the small intestine may be found the attachment of the stalk of the yolk sac (Pl. I-17) or its remnant (Pl. I-18) depending on the age of the chicken.

There is no line of demarcation between the colon and rectum, the two usually being considered as one, the rectum (Pl. I-21).

At the junction of the small intestine and rectum (Pl. I-20) the paired caecae (Pl. I-19) are given off anteriorly. Each terminates as a blind sac which is larger than the constricted part near its origin.

The rectum terminates in the anterior portion of the cloaca. There is no line of demarcation except a gradual widening.

The cloaca (Pl. I-22) is divided into three parts: the coprodaeum into which the rectum empties; the middle part, the urodaeum, into which the ureters and genital ducts enter; and the external part, proctodaeum, from which the bursa cloacae (Pl. I-23) extends antero-dorsally.

The anus (Pl. I-24) is comprised of a prominent dorsal and a ventral lip which meet at each side forming a lateral commissure.

The liver (Pl. I-15) is a two lobed organ lying posterior to the rudimentary diaphragm. Its posterior edges are quite noticeably notched. The gall bladder (Pl. I-14) is located on its visceral surface and from it the ductus cysticus carries the bile to the duodenum while a second duct, the ductus hepaticus comes directly from the left lobe and empties into the duodenum in close proximity to the first.

As already mentioned the main part of the pancreas (Pl. III-10, 11) is located in the loop of the duodenum. The pancreas is composed of three lobes, the dorsal and ventral lobes located as described and a third much smaller lobe extending dorsally to the spleen. Pl. III-12).

Measurements of the digestive tract of five chickens were made and are included in table 1, to show the relative lengths. By comparing the figures one may get an idea of the rate of growth.

TABLE 1. *Growth changes in the digestive tract*

Measurements	36 hr.	20 da.	5 mo.	1.5 yr.	2 yr.
Entire digestive tract	43 cm.	85 cm.	152 cm.	210 cm.	175 cm.
Angle of beak to crop	2.5 "	7.5 "	12.5 "	20 "	17.5 "
Angle of beak to proventriculus	5 "	11.5 "	20 "	35 "	27.5 "
Duodenum (complete loop)	6 "	12 "	20 "	20 "	25 "
Ileum and jejunum	24 "	49 "	85 "	120 "	92.5 "
Caeca	3.5 "	5 "	15 "	17.5 "	16 "
Rectum and cloaca	3 "	4 "	11.25 "	11.25 "	13.75 "

Orr (1931) took measurements and computed averages on the intestines of thirty-five birds. The birds ranged in weight from 1200 to 1800 gm., 85 per cent of which were within a 200 gm. range. His results follow: the duodenum (measurements including the entrance of the bile and pancreatic ducts) 25.3 cm.; the remainder of the small intestine 104 cm.; entire small intestine, 132.5 cm.; caeca, 14.4 cm.; and "colon" (measured from the entrance of the caeca to the point where the rectum began to widen) 6.36 cm.

Kersten (1912) made measurements on the intestine of twenty-four specimens ranging from 2 da. 3 hr. to 21 wk. 8 hr. (Table 2).

TABLE 2. *Length of the intestinal canal after hatching*

Week	Age Day	Hour	Length of small intestine up to yolk stalk	From yolk stalk to opening of caeca	Length of colon and rectum	Intestinal length with- out caeca	Length of a single caecum	Relative length of the caeca
	2	3	26.5*	17.25	3.25	47.5	4.5	0.19
	4	22.5	34.0	13.5	3.5	56.0	4.5	0.16
1		12	25.0	15.0	3.0	43.0	4.0	0.19
1	4	13	42.0	24.5	3.5	70.0	5.5	0.16
2		7			3.0		5.5	
2	3	16.5	27.0	18.0	3.0	58.0	4.5	0.15
3		4	43.0	29.0	4.0	76.0	6.5	0.17
3	3	15	37.0	25.0	3.5	65.5	4.0	0.12
4		4	41.5	24.0	3.5	69.0	6.5	0.19
4	3	15	54.0	37.0	4.0	95.0	4.75	0.10
5		2	31.0	21.0	3.5	55.5	5.5	0.20
5	4	13	40.0	23.0	4.0	67.0	5.0	0.15
6		7.5	43.0	20.5	4.5	68.0	5.5	0.16
6	3	13.5	49.0	29.5	4.5	83.0	6.0	0.14
7		21	52.0	59.5	5.5	117.0	11.5	0.20
7	4	16	52.5	30.75	4.25	87.5	7.0	0.16
8	3	5	65.0	49.5	5.5	120.0	12.0	0.20
9		6	70.0	54.0	6.0	130.0	10.5	0.16
10		10	60.0	36.5	4.5	101.0	9.5	0.19
11	1	7	55.0	45.5	5.0	105.0	6.5	0.12
12	2	6	57.0	35.75	5.25	98.0	9.0	0.18
13	3	3	71.5	56.0	5.5	133.0	11.5	0.17
15	5	8	75.0	43.75	6.25	125.0	14.0	0.22
21		8	77.0	50.5	9.5	107.0	16.0	0.23

* All measurements in centimeters.

In a three-week-old chick, Kersten found the length of the small intestine to be 72 cm., while the writer found it to be 61 cm. Likewise in a five-month-old specimen, Kersten found the length of the small intestine to be 127.5 cm. and the writer found it 105 cm. The latter measurement by Kersten compares with Orr's figures (132.5 cm.) of the intestinal length. An average of the length of the two adult specimens of the intestine studied by the writer was 128.7 cm. Other figures are similar: the duodenal length of the adult according to Orr was 25.3 cm. and the writer found 22.5 cm.; the length of the small intestine without the duodenum was 106.5 cm. according to Orr, and 140 cm. according to the author's observation; the caecal length as Orr gave it was 14.4 cm. and the author 16.5 cm. According to Kersten the caecal length in a five-month-old chicken was 16 cm. and

the author found it to be 15 cm. In the three-week-old specimen the figures for the caeca were similar, being 6.5 cm. as Kersten gave it and 5 cm. according to the author.

In the oldest specimen Kersten measured (21 weeks), the length of the "colon and rectum" was 9.5 cm. In the adult specimens, Orr found the "colon" to be 6.36 cm. in length

Magnan (1911a) made the statement that the total surface of the body was 2.4 times the intestinal surface in granivorous birds (917 individuals, 12 species). In the same article M. Caullery criticized Magnan's work and said that the surface of the intestine from a physiological viewpoint should be its glandular surface and should take into consideration the villi and crypts.

MATERIALS AND METHODS

The specimen material was secured from one carcass each of chickens aged thirty-six hours (male), twenty days (sex unrecorded), five months (female), one and one-half years (male), and two years (female). Besides these, livers and some additional material were obtained from two groups of baby chicks. Several specimens of the bursa cloacae were obtained from other chickens.

The following methods were used: paraffin embedding with the exception of frozen sections of a specimen of each liver; Harris hematoxylin and eosin were used as a routine stain; Weigert's elastic tissue stain was used for elastic connective tissue; Van Gieson's picro-acid-fuchsin was used for white fibrous connective tissue; frozen sections of liver were stained with Scharlach R [alcohol-acetone method according to Mallory and Wright (1924)]; mucin was demonstrated by Mayer's mucicarmine method as given by Hoepke (1930); keratohyalin granules were stained by Pasini's (1930) method; reticulum according to Foot and Menard (1927).

In the experiments with the baby chicks the chicks were killed at stated intervals and a section of the liver stained for fat to determine at what age the fat began to disappear and how long it persisted.

RESULTS

Observations were made on the digestive tract from the beak to the anus, including all appendages. No differences existed in the digestive tract of either sex, so the matter of sex will not be referred to again.

BEAK

The beak, as shown in Plate IV, figure A, consisted of three layers, bone (11), corium (6), and epidermis (1). The bone in the upper beak was the os incisivum and in the lower the os dentale. A layer of periosteum was observed outside the bone. (Pl. IV, fig. A-10).

The corium (Pl. IV, fig. A-6) extending from the periosteum to the epithelium was made up of connective tissue containing blood vessels (7), nerves (8), and taste corpuscles (9), as shown in Plate IV, figure A.

The epidermis (Pl. IV, fig. A-1) comprised four layers. The stratum germinativum (Pl. IV, fig. A-5) was composed of three rows of tall cylindrical epithelial cells which changed abruptly to the stratum granulosum. (Pl. IV, fig. A-4). This latter contained four to five layers of flattened cells, with their long axes parallel to the surface. They were distinctly

granular. Intercellular bridges were very prominent in this layer. The stratum lucidum (Pl. IV, fig. A-3) was less distinct than the corresponding layer in the skin of mammals. It comprised about one-eighth of the entire epidermis. The stratum corneum (Pl. IV, fig. A-2) was a very thick layer of flat structureless cells.

The general structure of the lower beak (Pl. IV, fig. B) corresponded to the above. The cutis appeared more vascular. There were no taste corpuscles present. The epidermis was about one-third as wide as that of the upper beak. The stratum germinativum contained polyhedral cells, instead of cylindrical cells as in the upper beak.

MOUTH CAVITY

The mucous coat of the mouth cavity was lined throughout with stratified squamous epithelium. Papillae from the tunica propria extended into its basal layers and projections from the epithelium protruded down between these papillae from the tunica propria. In the roof of the mouth (Pls. V-VII) the nuclei of the cells of the outer surface of the epithelium were flatter than those of the basal layer and took a deeper stain. In places they appeared to be in a small cavity which did not take any stain.

The division between the tunica propria (Pl. V, fig. A-2) and the submucosa (Pl. V, fig. A-3) was rather an arbitrary one since there was no muscularis mucosae present until the posterior part of the pharynx was reached. (Pl. VII, fig. A-3). The tunica propria contained many macroscopic papillae (Pl. VI, fig. A-3 and fig. B-6) which extended posteriorly. The microscopic papillae extending into the epithelium seemed shorter near the lateral borders. Elastic and white fibrous connective tissue was present in the tunica propria. Diffuse lymphoid tissue was observed in the tunica propria of the year-and-one-half-old specimen and a lymph nodule in the two-year-old specimen. These were not evident in young chickens.

The submucosa (Pl. V, fig. A-3) was considered as that portion deeply to the tunica propria in which salivary glands lie. Since one gland was practically in continuity with another and these glands were paired, there were few areas in which no glandular tissue was present.

The submucosa contained elastic and white fibrous tissue, the latter forming a capsule about the glands. A fatty cushion was observed deeply to the gland layers in many specimens. (Pl. V, fig. A-4). The muscular layer outside the submucosa was voluntary.

The floor of the mouth was very similar in structure to the roof of the mouth. Toward the lateral sides of the floor of the mouth the epithelium became less compact, the outer cells were more polyhedral, and the nuclei more spherical and pyknotic. The microscopic papillae of the tunica propria were more prominent.

TONGUE

The general structure of the tongue (Pl. VIII, figs. A and B and Pl. IX, fig. A) was similar in its entire length. A thick stratified squamous epithelium (Pl. VIII, fig. B-1) covered the dorsal surface. In some instances the surface was uneven, the projections resembling low blunt papillae.

The tunica propria (Pl. VIII, fig. B-2) comprised the second layer, which contained the anterior lingual salivary glands on either side, (Pl. VIII, fig. B-3), the entoglossal bone, (Pl. VIII, fig. B-4) (cartilaginous in young birds) in the middle, with voluntary muscle (Pl. VIII, fig. B-5)

below the bone. The tunica propria was made up of white fibrous and areolar tissue containing blood and lymph vessels and nerves. Prominent microscopic papillae of tunica propria extended into the epithelium. In old birds the lower surface contained diffuse lymphoid tissue and an occasional lymphoid nodule. No lymphoid tissue was present in the tongue of a young specimen. The stratified squamous epithelium of the ventral surface of the tongue (Pl. VIII, fig. B-6) was smooth. It was about one-third to one-fourth as thick as that of the dorsal surface. Toward the point of the tongue (Pl. VIII, fig. A) the lower epithelium became cornified and took a stain similar to the stratum corneum of the beak. Toward the base of the tongue the entoglossal bone (Pl. IX, fig. A-6) presented a different picture than in the mid-portion. Two wings of this bone extended latero-caudally just below the dorsal surface of the tongue. In the center the basihyal bone was observed. (Pl. IX, fig. A-5).

The muscles presented different pictures at different levels. Near the tip the muscle was practically absent. (Pl. VIII, fig. A). A cross section from the mid-portion of the tongue is shown in Plate VIII, figure B, and a section from near the base is shown in Plate IX, figure A.

SALIVARY GLANDS

Schauder's (1923) description and terminology was used for the location and naming of the salivary glands. A translation of the outline, including only the parts pertaining to the chicken, follows:

(a) Glands at the bottom of the oral cavity.

1. Anterior submaxillary: largely developed, paired glands in the angle between the lower rami of the maxilla.
2. Posterior submaxillary in group of 3:
 - a. anterior lateral, lying medial to the os dentale;
 - b. inter-mediare, caudoventral to a;
 - c. back mediale, postero-medial to and connecting with the intermediary group.

(b) Glands of angle of the mouth.

3. Angularis oris gland [Cholodkowsky (1892)]: lying in the angles of the beak, a small, three-cornered gland area.

(c) Glands of the tongue.

4. Anterior lingual: at the side of, in the middle of, and in the posterior part of the tongue.
5. Posterior lingual: on the dorsal surface of the base of the tongue.

(d) Glands of the roof of the mouth.

6. Paired glands joining medially in the hard palate lying before the posterior nares. [Maxillary of Heidrich, (1905)].
7. Medial and lateral palatine glands: extending longitudinally to the posterior nares.
8. Sphenopterygoid: in the roof of the pharynx.

(e) Glands of the pharyngeal canal.

9. Criccoarytaenoideae: lying lateral to the larynx in the sub-mucosa of the cutaneous mucous membrane.

The salivary glands all presented the same structure. They consisted of branched tubular glands with openings into a common cavity (Pl. X, fig. A-3) from which an excretory duct (Pl. X, fig. A-1) led to the mouth cavity. The angularis oris and the maxillary glands had a single opening

for each gland. Others had many openings for a single gland. The cells were columnar in shape with small nuclei which lay close to their bases. Fine septa containing white fibrous and elastic fibers, capillaries and some muscle fibers extended between the tubules from the capsule which surrounded the acini. Basket cells were not definitely identified. There were some large cells in the septa but their nature was not determined. The glands were entirely composed of mucous cells and in no case were serous cells observed.

The buccal epithelium extended into the duct a short distance and then changed to a low columnar type which continued into and lined the collecting cavity, becoming taller again in the latter.

Lymphoid tissue (Pl. XI, fig. A-4 and Pl. XII, fig. B-6) was found between the lobules of all glands of the adult specimens except in the anterior lingual. Only the third group of the posterior submaxillary and the cricoarytenoid contained lymphoid tissue in the five-month-old specimen and of the two younger specimens the only indication of lymphoid tissue was in the cricoarytenoid of the thirty-six-hour chick.

A peculiar structure was observed in some of the glands. The cell outlines had disappeared and the result was one conglomerate mass of secretion and cellular debris. (Pl. XI, fig. A-5). A similar area in another section (Pl. XI, fig. B-2) took a mucous stain.

PHARYNX

The pharynx extended from the row of papillae at the back of the hard palate (Pl. VI, fig. A-3) to the row of papillae at the entrance of the esophagus. (Pl. VII, fig. A-8). The roof of the pharynx was composed chiefly of the medial palatine (Pl. VI, fig. C-4) and the sphenopterygoid glands (Pl. VI, fig. C-5) between which lay a voluntary muscle making an oblique angle with the epithelium. (Pl. VI, fig. C-3). The microscopic structure resembled that of the mouth as did that of the floor of the pharynx. The latter contained the posterior lingual and the cricoarytenoid glands in its wall.

The structure regarded by some authors as a tonsil was observed as a lymphoid infiltration of the tunica propria in the region of the aditus laryngis. (Pl. IX, fig. B). Some lymph nodules were present under the stratified squamous epithelium.

The wall of the digestive tube proper, consisted essentially of a mucous membrane, comprised of an epithelial lining, tunica propria and muscularis mucosae; a submucosa; a lamina muscularis; and an adventitia or serosa depending on the location of the organ.

ESOPHAGUS

The esophagus was similar in structure both anterior and posterior to the crop. It was characterized by a wide stratified squamous epithelial layer. (Pl. XIII, fig. A-1). The basal layer of the epithelium projected between prominent papillae of the tunica propria. The epithelium showed a loosening of the outer layers with a tendency to slough off. In the tunica propria (Pl. XIII, fig. B-2) were contained large mucous glands. (Pl. XIII, fig. B-3). The tunica propria was made up of a network of fibrous tissue which contained many blood vessels, lymph vessels, and nerves. As the bird advanced in age the elastic tissue became more dense in the submucosa, (Pl. XV, fig. B) and was observed in the tunica propria. (Pl. XV, fig. A). Lymphoid nodules were also observed. The mucous glands were

lined by low cuboidal epithelium which decreased in height as it approached the surface finally becoming flattened. (Pl. XIV, fig. B-3).

The muscularis mucosae (Pl. XIII, fig. A-4) was of thick involuntary muscle arranged longitudinally. It was about three times as thick as the outer longitudinal layer of the lamina muscularis.

The submucosa (Pl. XIII, fig. A-5) was thin, hardly discernible in places, while in others it widened out and a few blood vessels and nerves could be distinguished in it.

The lamina muscularis (Pl. XIII, fig. A-6 and 7) consisted of a thick inner circular layer and a thin outer longitudinal layer of involuntary muscle. In specimens from birds aged five months, one and one-half years, and two years, a heavy elastic tissue layer was in close contact with the outer longitudinal muscle layer.

The outer layer or adventitia (Pl. XIII, fig. A-8) was thin, and served to unite the esophagus to adjacent structures. It contained elastic and white fibrous tissue and many plexuses of blood and lymph vessels and also nerves.

CROP

The lesser curvature of the crop (Pl. XVI, fig. B) had essentially the same structure as the esophagus of which it was a part. The structure of the diverticulum of the crop (Pl. XVI, fig. A) differed in some respects from the esophagus with which its walls were continuous. The glands of the crop (Pl. XVI, fig. B-3) were confined to an area which was close to the junction with the esophagus. The epithelial projections between the papillae of the tunica propria were more rounded. The same sloughing of the epithelium was observed here. None of the specimens showed any lymphoid tissue in the diverticulum of the crop but it was present in the esophageal wall of the crops of older birds. In three specimens the muscularis mucosae appeared to be arranged in an outer longitudinal and an inner circular layer. (Pl. XVI, fig. A-3). In the other two birds it was difficult to make out any circular layer. Elastic tissue was observed exteriorly to the outer longitudinal muscle layer of this organ in the baby chick and it increased with age, spreading to the other layers of the wall. Many blood vessels were present in the adventitia, between the muscle bundles, and in the submucosa.

JUNCTION OF PROVENTRICULUS AND ESOPHAGUS

The epithelium of the esophagus became narrower as it approached the proventriculus and changed at the junction into the one-layered simple columnar epithelium found in the remainder of the digestive tract except in the anus.

There was no abrupt change from one type of gland to the other. The mucous glands of the esophagus were found in the inner layer of the tunica propria while in the deeper layer the anterior extremity of the glands of the proventriculus was observed.

Lymphoid tissue was found in the tunica propria of a section taken from a three-day-old bird.

PROVENTRICULUS

The proventriculus was lined by simple columnar epithelium, which formed the simple tubular glands of the surface between which opened the excretory ducts of the deeper glands. (Pl. XVII, fig. A-4). The tunica

propria, (Pl. XVII, fig. A-3) the connective tissue layer below the epithelium, was thrown into small projections due to the size of the large multi-lobular glands (Pl. XVII, fig. B-3) which were contained in the deeper part of it. The muscularis mucosae (Pl. XVII, fig. B-4) was observed below the surface glands, in the septa between the deeper glands, and in a longitudinal layer almost in contact with the lamina muscularis. The submucosa (Pl. XVII, fig. A-8) was so thin as to appear absent in places. The lamina muscularis (Pl. XVII, fig. A-9 and 10) showed the usual outer longitudinal and inner circular layers. The adventitia (Pl. XVII, fig. A-11) appeared as a loose fascia containing few blood and lymph vessels and nerves.

Elastic tissue was demonstrable in the thirty-six-hour chick and increased in amount as the bird aged. It predominated in the tunica propria and the septa between the deep glands. Lymphoid tissue was observed in the tunica propria in the three oldest specimens.

The deeper glands of the proventriculus presented two different pictures. On cross section the gland tubules (Pl. XVIII, fig. A-1) showed a meshwork, the strands of which had a serrated appearance. On longitudinal section the gland tubules (Pl. XVIII, fig. A-2) showed long tubules, the cells (Pl. XVIII, fig. A-4) of which were arranged obliquely to the axis of the tubule. The distal half or more of a single cell was not in contact with the neighboring cell thus giving the appearance of a serrated edge. A spherical nucleus was situated about the central part of the cell usually located toward the proximal half. The tubules opened into a central collecting cavity (Pl. XVII, fig. A-5) which was lined with columnar epithelium. These collecting cavities opened on the inner surface of the proventriculus as mentioned above.

The septa (Pl. XVIII, fig. A-3) surrounding the lobules contained white fibrous and yellow elastic connective tissue, some muscle fibers, blood and lymph vessels, and nerves. The surface epithelium, the epithelium of the collecting canals, and of the excretory ducts of the deeper glands, took a mucous stain in its distal third.

JUNCTION OF PROVENTRICULUS AND GIZZARD

The deep glands of the proventriculus ended abruptly, followed by an increase in the length of the tubular glands of the surface. These soon took on the characteristic aspect of the gizzard glands and a keratinized layer was observed above them.

The white fibrous connective tissue of the tunica propria joined with that from the submucosa and continued into the gizzard as the submucosa.

The layer of the muscularis mucosae above the deep glands of the tunica propria apparently tapered off at the point where the tunica propria and submucosa joined. The deep portion of the muscularis mucosae widened out and was continued into the gizzard with the circular muscle layer of the proventriculus. These two continued as separate layers for a short distance and then became fused into a single layer of fibers which became a part of the musculari intermedii of the gizzard.

The outer longitudinal layer of the lamina muscularis ceased at the junction of the proventriculus and the gizzard.

Elastic tissue was particularly dense in this region in the two-year-old specimen. (Pl. XVIII, fig. B).

GIZZARD (VENTRICULUS)

The gizzard had as its innermost lining a horny layer (Pl. XIX, fig. A-1) which was about three-fourths as thick as the glandular layer adjacent to it. This horny layer was an exudate from the glands and contained wavy lines (Pl. XIX, fig. B-2) parallel to the surface, and colonnades or thickenings (Pl. XIX, fig. B-3) perpendicular to the surface. The former apparently were formed by consecutive layers of the exudate and the latter by secretion being poured out at the same point; also, cellular debris was observed in this horny layer.

The epithelium of the mucous membrane was simple columnar and contained crypts, at the bottom of which opened the tubular glands (Pl. XIX, fig. A-2) of this organ.

The glands of the gizzard were in the tunica propria and arranged in groups presenting in longitudinal section the arrangement shown in Plate XXI, figure A-1. The gland tubules were lined with low cuboidal epithelium containing spherical nuclei which bulged into the lumen in places. (Pl. XXI, fig. B-3). The tubes were filled with an exudate which took a bright red stain with keratohyalin staining. (Pl. XX, fig. B-1). The gland cells themselves contained small granules of keratohyalin. (Pl. XX, fig. B-3). Elastic tissue was demonstrable in the tunica propria of the three oldest birds.

The muscularis mucosae was absent from the gizzard. The submucosa (Pl. XIX, fig. A-3) was a dense layer of white fibrous and yellow elastic connective tissue, the former predominating. Blood vessels, lymphatics and nerves were present.

The muscular mass (Pl. XIX, fig. A-4) was comprised of a single thick layer of parallel fibers which extended from one aponeurosis to the other.

Near the center of the tendinous aponeurosis (Pl. I-11) the submucosa came in contact with the tendinous tissue of the aponeurosis and the muscular tissue was absent.

Exterior to the muscle a thin layer of connective tissue (Pl. XIX, fig. A-5) containing nerves and blood and lymph vessels, was present. Elastic tissue was present in this layer in all birds observed. Peritoneum covered the whole organ.

Lymphoid tissue was not observed in the gizzard wall in any of the five specimens studied.

SECTION BETWEEN GIZZARD AND DUODENUM

In the region between the gizzard and the duodenum, the mucous membrane became narrow only to widen again after it made an acute angle. It again made a slight turn and at this point the horny layer ceased. Just posterior to this there was a short section resembling the portion of the mammalian duodenum in which Brunner's glands are present. Lymphoid tissue was observed in the area between the gizzard and duodenum in specimens as young as three days old.

SMALL INTESTINE

The structure of the small intestine, duodenum included, was similar throughout. The inner layer of the mucous membrane was lined with simple columnar epithelium with many goblet cells. These were mucous both on the lumen and in the glands of Lieberkühn. (Pl. XXIV, fig. B-4).

The inner surface showed villi (Pl. XXVI, fig. A-10) between which the crypts of Lieberkühn (Pl. XXVI, fig. A-8) opened. The villi contained lacteals, blood vessels, muscle fibers, and lymphoid tissue, the latter varying with the age of the chicken. In the thirty-six-hour chick there was much embryonic connective tissue in the tunica propria filling the villi and surrounding the glands of Lieberkühn. Practically no lymphocytes were observed at this age but by the twentieth day they were scattered throughout the tunica propria. The villi branched, sometimes twice. (Pl. XXII, fig. A). Elastic tissue was observed in the tunica propria of the three oldest chickens. (Pl. XXIII, fig. B-1).

The muscularis mucosae was comprised of an outer circular and an inner longitudinal layer. (Pl. XXVI, fig. A-5). The latter sent fibers into the villi. In places the outer circular layer appeared to fuse with the circular layer of the lamina muscularis. (Pl. XXVI, fig. A-6).

The submucosa was apparent only in a few places and then was only a very thin layer. (Pl. XXVI, fig. A-4). There were a few blood and lymph vessels and nerves in addition to the connective tissue.

The lamina muscularis was made up of an inner circular (Pl. XXVI, fig. A-3) and an outer longitudinal (Pl. XXVI, fig. A-2) muscle layer with a connective tissue layer on each side, which contained plexuses of nerves, and blood and lymph vessels.

The subserous layer (Pl. XXVI, fig. A-1) was very thin, consisting of both white fibrous and yellow elastic fibers. (Pl. XXIII, figs. A and B). Blood vessels, lymph vessels and nerves were contained in its meshes. It was limited outside by the peritoneum.

Diffuse lymphoid infiltration of the tunica propria and a few small lymph nodules were observed in the five-month-old specimen, and in the two-year-old bird the nodules were so numerous at one place in the small intestine as to appear almost like Peyer's patches. (Pl. XXII, fig. B-5). A nodule was observed in the circular muscle layer of intestine in the one-and-one-half-year-old specimen.

At a point near the end of the duodenum the pancreatic and bile ducts entered. (Pl. XXV, fig. A-2). There was an elevation in the mucous membrane of the duodenum at this point.

The villi of the duodenum were the longest in all cases. With the exception of the thirty-six-hour chick the diameter of the small intestine diminished from the duodenum to the rectum. In the thirty-six-hour chick the diameter of the duodenum was not so large as the anterior half of the small intestine. In this portion the villi were wider and shorter, even appearing leaf-like in some places. Toward the posterior portion the villi increased in length again but the tube decreased in diameter becoming even smaller than the duodenum.

A circular sphincter muscle was observed at the entrance of the small intestine into the rectum. (Pl. XXVI, fig. B-3).

CAECA

The muscular coats of the caeca were continuous with those from the small intestine and rectum. The general structure of the caeca may be briefly summarized at this point. The structure of the different portions will be discussed later. A mucous membrane lined with columnar epithelium (Pl. XXVII, fig. A-1) containing goblet cells; villi in varying lengths depending on the region (Pl. XXIX, fig. B-3 and Pl. XXVII, fig. A-2); a

muscularis mucosae (Pl. XXVII, fig. A-4) absent in places; a submucosa (Pl. XXVII, fig. A-5) of white fibrous and yellow elastic tissue containing nerves, and blood vessels and lymph plexuses; a lamina muscularis (Pl. XXVII, fig. A-6 and 7) varying in thickness and arrangement, and a serosa (Pl. XXVII, fig. A-8) rich in nervous elements.

The caeca presented three different pictures depending on whether the proximal, middle, or distal portion was being considered. In Plate XXIX, figure B-3, even the proximal portion presented two slightly different views, because of a difference in contraction.

In this portion were prominent villi. They had a structure similar to those of the small intestine. The muscularis mucosae and submucosa were both thin layers and crowded close to the base of the villi. The lamina muscularis was marked by a thick inner circular layer and a thin outer longitudinal layer. No lymphoid tissue was observed in the section of a thirty-six-hour chick. In a section similarly cut, from a caecum of the twenty-day-old chick, one small area of lymphoid tissue was seen. In a caecum of a five-month-old specimen cut at the same proximal level, the tunica propria was a mass of lymphoid tissue with several nodules. A longitudinal section from the one-and-one-half-year-old specimen showed an extensive area just anterior to the origin of the caeca which was completely infiltrated with lymphoid tissue (Pl. XXVIII, fig. A-4) and contained numerous nodules (Pl. XXVIII, fig. A-5).

In the mid-portion, the villi were shorter and broader. (Pl. XXVII, fig. A-2 and fig. B-2). Here again the constriction of the wall resulted in a slightly different picture. In a constricted part of the mid-portion the villi were longer, the muscle thicker, and the whole circumference smaller than in a dilated portion at the corresponding level. Plica circulares were present at this level.

The muscularis mucosae contained a distinct inner circular and an outer longitudinal layer in the thirty-six-hour specimen. No other showed this arrangement definitely. Lymphoid tissue became present with advanced age.

Near the blind end of the caeca of the thirty-six-hour chick the inner circular and outer longitudinal muscular layers were nearly the same width. True villi were not present. Many eosinophils were present in the tunica propria. The muscularis mucosae was absent in places. Goblet cells were present in the epithelium. No lymphoid tissue was present.

In the distal portion of the caeca of a twenty-day chick the inner circular muscle had increased to about three times the width of the longitudinal muscle. The surface of the mucous membrane approached a villi-like arrangement between the plica circulares. On the plica themselves the villi appeared as blunt projections. Eosinophils were numerous in the tunica propria. The muscularis mucosae comprised an inner circular and an outer longitudinal layer. It was present at all points. Goblet cells were observed in the epithelium. Much diffuse lymphoid tissue was present. The blind end of the caeca of the five-month specimen was like the above with many lymph nodules in addition. The one-and-one-half and two-year-old specimens showed the same structure as the five-month specimen.

RECTUM

The rectum (Pl. XXIX, fig. A) as a whole resembled the small intestine. Villi were present in all specimens. Scattered lymphocytes were

observed in the tunica propria of the thirty-six-hour and twenty-day-old chicks, and lymph nodules in the older specimens.

CLOACA

The cloaca was separated from the rectum by a slight constriction (Pl. XXX, fig. B-5) of the circular muscle forming a somewhat circular orifice. It was not visible from the exterior of the rectum. The cloaca was divided into three parts (Pl. XXX, fig. B), coprodaeum (2); urodaeum (3); and proctodaeum (4), by transverse folds. The dorsal fold (Pl. XXX, fig. A-2). The ureters and genital tracts opened on the floor of the to the bursa which was in the dorsal wall of the proctodaeum (Pl. XXX, fig. A-2). The ureters and genital tracts opened on the floor of the urodaeum.

All three parts had a similar structure. Villi were present. They were finger-like in the coprodaeum, but became more leaf-like and decreased in height in the urodaeum and proctodaeum.

The cloaca was lined with columnar epithelium which extended as far as the anus. There were plica circulares present in addition to the folds between the compartments. Lymphoid elements in the tunica propria and elastic tissue throughout the wall increased with the age of the specimen.

ANUS

The anal opening (Pl. XXXI, fig. A-9) was lined with stratified squamous epithelium. The tunica propria at this point contained many papillae. After turning on the inside of the lip the epithelium became thinner, papillae of the tunica propria were absent, and by the time it reached the fold it had become the columnar epithelium of the proctodaeum. (Pl. XXXI, fig. B-4).

The muscularis mucosae was absent in the anus, and the tunica propria and submucosa were fused into a thin, loosely arranged connective tissue layer. (Pl. XXXI, fig. B-2).

Just anterior to the bursa cloacae a voluntary muscle began. It extended as a circular muscle to a point in the wall of the proctodaeum just above the ventral lip of the anus. (Pl. XXXI, fig. A-4). Here the inside portion of the circular layer began to arrange itself in a longitudinal direction. The two portions were continued thus for a short distance. By the time the lamina had reached the furthestmost point of the dorsal lip of the anus its few fibers were all arranged longitudinally. (Pl. XXXI, fig. A-5).

The muscular arrangement of the ventral lip was a little different. The fibers were circular as above, then a few fibers of the inside portion changed to a longitudinal direction (Pl. XXXI, fig. A-6) only to change back to a circular arrangement at the extremity of the ventral lip. (Pl. XXXI, fig. A-8).

LIVER

The livers of the five specimens presented one variation,—the fat spaces in the liver cells of the baby chicks. This variation will be discussed later. The liver of the chicken differed little from that of the mammal. The interlobular septa were probably less apparent than those of the domesticated animals. The portal canal (Pl. XXXII, fig. B) contained the portal vein (1), lymph vessel (4), hepatic artery (3), and bile ducts (2). The interlobular veins were not prominent.

The central veins (Pl. XXXII, fig. A-1) were distinguished by the prominent sinusoids which entered them. They were lined with a thin endothelial membrane. (Pl. XXXII, fig. A-2). The sinusoids were also lined with endothelial cells. The Kupffer cells were definitely marked.

The liver epithelium was arranged in a tubule of four to six cells about an intralobular bile capillary. The tubules were well marked. The liver cell was a pyramidal cell with its apex bordering the lumen of the tubule. A large spherical nucleus was in the distal half of the cell.

Elastic tissue was confined to the walls of the blood vessels, to the connective tissue septa surrounding them, and to the capsule of Glisson surrounding the liver. White fibrous tissue was distributed similarly.

The cords of liver cells (Pl. XXXII, fig. A-3) were supported by a meshwork of reticular tissue. (Pl. XXXIII, fig. A-2).

In the work on the livers of baby chicks a change in the color was observed at about the fifteenth day. Microscopically a slight decrease in the amount of fat was observed by the twelfth day (Pl. XXXIV, fig. B). By the fifteenth day a considerable decrease was noticeable in the amount of fat (Pl. XXXIV, fig. C) and it continued to decrease until on the twenty-first day (Pl. XXXIV, fig. E) the fat globules were confined to a small area about the central veins. This condition persisted until about the twenty-fifth day. After this time occasional fat droplets were found scattered throughout the liver. The oldest specimen observed was forty-five days old. The adult hematoxylin-eosin-stained specimens showed an occasional vacuole in the liver cells which may have been fat. There were indications that the type of feed and the relation of time of feeding to time of slaughter would change this picture. This phase was considered beyond the scope of this study.

BILE DUCT

The wall of the bile duct consisted of the following layers: an outer connective tissue serosa; a lamina muscularis with an outer longitudinal (incomplete) and inner circular layer; a thin submucosa containing blood vessels, lymph vessels and nerves; an incomplete muscularis mucosae, sometimes with both circular and longitudinal layers present, other times only a circular; and a tunica propria extending into villi lined with cylindrical epithelium. (Pl. XXXV, fig. A).

GALL BLADDER

The gall bladder had a thick vascular serosa on the outside. Between the serosa and the mucous membrane there was a thin layer of muscle extending longitudinally. The mucous membrane was a loose connective tissue layer thrown into villi-like folds lined with columnar epithelium.

PANCREAS

The pancreas was a lobulated tubulo-acinar gland, the interlobular septa being very indistinct. The structure of the pancreas consisted of many acini (Pl. XXXVI, fig. A-1 and fig. B-1) which emptied into small collecting ducts lined with flattened epithelium. These ducts in turn emptied into larger ones lined by cuboidal epithelium (Pl. XXXVI, fig. A-3) and so on until the large collecting ducts (Pl. XXXV, fig. B) with columnar epithelium were reached.

The acinar cells of the pancreas were low columnar and wider at their base than near the lumen. They had a granular cytoplasm, denser near the lumen. The granules were probably similar to the zymogen granules of the mammalian pancreas although the eosin staining was more uniform throughout the cell. The spherical nucleus was in the basal half of the cell. Centro-acinar cells were demonstrable.

Islets of Langerhans (Pl. XXXVI, fig. A-2) similar to those of the mammalian pancreas were observed. They were lighter staining cellular structures distributed throughout the pancreatic tissue. The islet tissue was not separated from the rest of the pancreas by a connective tissue layer. There were small amounts of reticular tissue present in the islets. Elastic and fibrous tissue was confined to the blood vessels and ducts and vicinity, and to the peritoneal covering. Plate XXXVI, figure B shows reticular tissue in the pancreas.

PANCREATIC DUCT

The structure of the pancreatic duct was identical to that of the bile duct. Its wall was slightly thicker. (Pl. XXXV, fig. B).

BURSA CLOACA

The wall of the bursa cloacae consisted of a thin serosa comprised chiefly of white fibrous connective tissue, an outer circular and an inner longitudinal involuntary muscle layer, and a mucosa thrown into longitudinal folds with a structure characteristic of this organ alone.

Some of the muscle fibers joined with white fibrous connective tissue and some with elastic fibers to form a trabecula (Pl. XXXVII, fig. A-1) which extended the length of the fold and sent septa in between the lymph follicles (Pl. XXXVII, fig. A-2). This trabecula was rich in blood vessels.

There were many follicles in a fold. The follicle was a dense lymphocytic structure which was divided into a cortical (Pl. XXXVII, fig. B-4) and a medullary (Pl. XXXVII, fig. B-3) portion, the latter being less dense, comparable to the germ center of a lymph node. This medullary portion was in contact with the columnar epithelial lining. This epithelium was pseudostratified columnar on and near the tips of the folds. In between the folds it appeared as simple tall columnar, although there were areas in which it appeared cuboidal. Goblet cells were present. The medullary portion extended out through the cortical portion to join with an indipping in the epithelium (Pl. XXXVII, fig. B-2). The cortical portion was set off from the medullary portion by a reticular network (Pl. XXXVIII, fig. A-3) and by a row of cells which appeared similar to a columnar epithelium in places. No blood vessels were observed in the medullary portion but were present in the cortical part.

YOLK SAC

The wall of the yolk sac consisted of a fibrous connective tissue layer (Pl. XXXIX, fig. B-4) upon which were located many longitudinal folds of columnar epithelium containing many vacuoles. (Pl. XXXIX, fig. B-2). The whole was surrounded by a serous membrane. (Pl. XXXIX, fig. B-5).

YOLK STALK

The wall of the yolk stalk was similar to that of the intestine with which it was continuous. A lamina muscularis, comprised of an outer and an inner circular muscle (Pl. XXXIX, fig. A-2), and a longitudinal muscularis mucosae with both longitudinal and circular fibers (Pl. XXXIX,

fig. A-4) made up its muscle. The submucosa was thin. (Pl. XXXIX, fig. A-3). The tunica propria (Pl. XXXIX, fig. A-5) was a dense connective tissue layer arranged in villi-like projections which were lined with a simple columnar epithelium. (Pl. XXXIX, fig. A-6). Few goblet cells were observed.

In the thirty-six-hour chick no lymphoid tissue was observed but in the one-and-one-half-year-old specimen it resembled the proximal part of the caeca, the lymphoid tissue was so large in amount.

Another section taken from a laboratory dissector of unknown age (adult) showed a wall of four layers; the inner, a columnar epithelial layer (no folds or crypts); the second, a lymphoid layer which had obliterated the tunica propria; the third, a thick circular muscle layer, and the outer, the serosa.

DISCUSSION

According to Krause (1922) there are four layers in the beak but the author observed only three. The corium consisted of one layer and was not divided into two as Krause described it.

No evidence of teeth was found, thus agreeing with previous authors.

A hard palate was present but no soft palate was observed. This was in agreement with Heidrich (1905) and Ward and Gallagher (1927).

There was no microscopic line of demarcation between the mouth and pharynx but the last row of papillae on the hard palate and those at the base of the tongue seemed to divide these two cavities. If one considers these as boundaries then it may be stated that there is an exact line of demarcation between the mouth and the pharynx. Grossman (1927), Heidrich (1905), and Bradley (1915), used these as convenient marks for separating the two. The author agrees with Heidrich that the muscularis mucosae began in the posterior part of the pharynx but cannot agree with him on the musculature of the pharynx. Heidrich (1905) stated that the pharynx had no muscle. A thick muscle inserted itself obliquely in the wall of the pharynx.

Taste corpuscles were observed only in the beak. Krause (1922) found them in both the beak and the tongue.

A tonsil as such is not regarded as a structure belonging to the chicken. There was present only a lymphocytic infiltration of the tunica propria with some lymph nodules present. This was observed only in adult birds and was particularly prominent in the region of the aditus laryngis. Killian (1888) described a tonsil in the region of the Eustachian tubes.

Schauder's (1923) classification of the salivary glands was followed. The structure of these glands was found to be similar, in agreement with Kovacs (1928). Heidrich (1905) found basket cells, while Holting (1912) did not. The author did not definitely determine whether basket cells were present or not. Heidrich studied also the changes taking place in the gland in the physiologic state, but this was not considered in this paper.

In agreement with Barthels (1895), Batt (1925), Bradley (1915), Browne (1922-23), Heidrich (1905), Kaupp (1918), and Kovacs (1928), the outer layer of the lamina muscularis of the entire digestive tract (except the gizzard) was longitudinal.

The esophageal tonsil of Kovacs (1928), Zietschmann (1911), and Schauder (1923), was not observed. There are four layers in the wall of

the esophagus as Batt (1925), Grossman (1927), and Marschall (1895), have agreed. The detachment of the surface layers of the mucosa, as observed by Barthels (1895), was also observed by the writer.

The author found that the crop had the same general structure as the esophagus. In agreement with Barthels (1895) no glands were found in the diverticulum of the crop but were confined to the esophageal wall.

The glands of the proventriculus were multilobular. Schreiner (1900) and Zietschmann (1911) also described them as multilobular. No evidence was found that would lead one to say that there was a variation in size of the glands in different regions of the proventriculus. Wilezewski (1870) thought they were larger at the esophageal end and smaller toward the gizzard, and Marschall (1895) found them to be small in size at both extremities. There was some variation in the size of the lobules but this may have been because of the way they were cut. It is doubtful if one should try to compare the glands of either the proventriculus or the gizzard to regions in mammalian stomachs, as did many authors, because the variation is too great.

There were reasons to support Bradley's (1915) and Zietschmann's (1911) idea that the deep glands were beneath the muscularis mucosae and that the lamina muscularis had three layers, because of the fact that there was much connective tissue about the glands. This could easily be taken for the submucosa as there was such a thin layer of connective tissue between the two inside muscle layers. However, there were fibers from the inner longitudinal layer which coursed in between the glands, hence, the inner longitudinal muscle layer was considered the muscularis mucosae. This is in agreement with Batt (1925).

The section of the gut between the proventriculus and gizzard was characterized by the lack of deep proprial glands as Schauder (1923), Zietschmann (1908), Kovacs (1928), Hässe (1866), and Cazin (1886b) described. The tubular or superficial proprial glands were also longer as Cazin (1886a) stated.

There was little disagreement on the structure of the mucous membrane of the gizzard and the findings in this study agreed with those of previous authors. However, elastic tissue was not confined to an area beneath the gland region alone as Zietschmann (1911) said, but was also found in the tunica propria of adult specimens and in the subserous layer. The thin outer longitudinal muscle which Batt (1925) described was not observed in any specimens studied.

Neither the fold described by Marschall (1895) and Kaupp (1918), nor the valve mentioned by Otte (1928), was observed between the gizzard and the duodenum.

A thin submucosa was found in the small intestine. This finding was in contrast to Cloetta's (1893) idea that the submucosa was absent and that the blood and lymph vessels were in the tunica propria. The outer layer of the muscularis mucosae was so intimately associated with the circular layer of the lamina muscularis that the submucosa was not discernible in places.

The two bile and three pancreatic ducts entered through a papilla as described by Gadow (1879).

There was evidence that the structure of the villi changed with age in accordance with Bujard's (1906) observations, because the villi of the thirty-six-hour chick had a somewhat different aspect than that of all the other specimens studied. The villi were leaf-like in some parts of the small

intestine at this age. No observations were made on the geometric regularity of the villi described by Clara (1927b).

The position of goblet cells in birds of different ages as Cloetta (1893) described, was not studied, nor was any consideration given to the cells of Paneth. Greschik (1922) and Clara (1926b and 1927a), found them, while Cloetta doubted their presence.

The writer agrees with Retterer and Lelievre (1910a) that areas were present which had the appearance of Payer's patches but that no true Peyer's patches, as described by Otte (1928), were present.

Sufficient observations were not made to prove that lymph nodules were more abundant in one region of the intestine than another, only that they were observed in all sections from adult specimens studied. Batt (1925) found more lymph nodules in the duodenum than in the remainder of the small intestine.

The author agrees with Cloetta (1893) and Otte (1928) that the duodenum had the widest lumen of the small intestine, except in the thirty-six-hour chick, in which the small intestine just beyond the duodenum was wider.

An iliac sphincter as described by Zietschmann (1911) was observed.

Much lymphoid tissue was found in the mucosa of the caeca of all birds studied except in the thirty-six-hour chicks; and as Looper and Looper (1929) described, there were many lymph nodules. The lymphoid area was observed in the proximal portion of the caeca as described by several authors. [Bradley (1915), Kaupp (1918), Oppel (1897), and Zietschmann (1911)].

Observation did not bear out the findings of Batt (1925) that the muscularis mucosae was well developed, but agreed with Looper and Looper (1929), that it was absent in many places.

The blind ends of the caeca presented such a varied structure in different specimens that one can agree with either Browne (1922-23) and Zietschmann (1911), that the villi were short or absent; or with Batt (1925), that the mucous membrane was thrown into folds having the appearance of villi.

No particular attention was given to the goblet cells except that they were present. Zietschmann (1911) stated that they were lacking where lymphoid tissue was plentiful.

Observations bore out the statement of Looper and Looper (1929) that lymphoid tissue infiltrated the caeca with increasing age.

The rectum was similar in structure to the small intestine. The findings agreed with those of Greschik (1912) that the villi were the same height as those of the small intestine; that lymphoid tissue was present; and that the submucosa was weakly developed and in places not discernible.

The rectum was separated from the cloaca by a constriction in the circular muscle which might be termed a sphincter according to Bütschli (1924), or a valvular circular orifice, according to Otte (1928), and Owen (1866).

The cloaca was divided into three compartments. Ward and Gallagher (1927), Thomson (1923), Gadow (1891a), Bütschli (1924), Schauder (1923), and Bradley (1915), found the same.

The writer agreed with Marschall (1895) that the anal opening was a horizontal slit.

This study agreed with previous studies of Krause (1922) and Batt (1926), in that the chicken liver is similar to the mammalian liver. Portal

systems and central veins were observed. Batt (1928) described central veins but Zietschmann (1911) stated that they were lacking. Elastic fibers were found in the capsule of Glisson in addition to the vessel walls as Zietschmann observed them, and a reticulum was observed but not "scant" as Batt (1926) stated. Observations made on the color of the liver did not agree with those of Doyle and Mathews (1928) who stated that the liver changed from a yellow color to a moroon color at the age of one week to ten days. This change was found to take place at approximately fifteen days of age.

In specimens of gall bladder observed, no outer longitudinal muscle layer was seen in the wall, as Zietschmann (1911) stated, since only one, a circular layer, was present.

Studies on the pancreas substantiated Clara's (1923-24) work in that the pancreas had three lobes, dorsal, ventral and splenic; and that the splenic lobe had no separate excretory duct. The "pseudo-islet" of Clara was not observed. The writer agreed with Böhm (1904) that the islets of Langerhans were not set off from the surrounding tissue by connective tissue. Centro-acinar cells were found, in agreement with Krause (1922) and Zietschmann (1911).

The pancreatic ducts were similar to the bile ducts as Zietschmann (1911) stated.

Observations made on the remnant of the yolk stalk indicated that it is constantly present as Muthmann (1913) and Latimer (1924) thought. It is a continuation of the intestine, and becomes degenerated with age as Maumus (1902) observed.

The writer found a thin serosa in the bursa cloacae as did Osawa (1911). Involuntary muscle was present in its walls as Jolly (1915) and Gadow (1891a) observed. This muscle presented an outer circular and inner longitudinal arrangement as Osawa (1911) found. Retterer (1885) found the opposite. No blood vessels were observed in the medullary portion. Schumacher (1903) and Wenkebach (1889 and 1896) also made this observation. Schumacher's (1903) observations that the epithelium varied from cuboidal, to tall columnar, to pseudostratified types, were verified. Goblet cells were present. Gadow (1891a) found them but Wenkebach (1889) did not.

A valve was present over the opening into the bursa cloacae. Forbes (1877) could not find one while Retterer (1885) observed one in *Uria troile*.

SUMMARY

A microscopic study of the digestive tract with its appendages was made on chickens of different ages.

The general structure of the wall was as follows: a mucous membrane comprised of an inner epithelial lining, a tunica propria and a muscularis mucosae; a thin submucosa; a lamina muscularis with an inner circular muscle layer and an outer longitudinal muscle layer; and an outer adventitia or serosa depending on the location of the organ.

The epithelium of the mouth contained many epithelial papillae which projected posteriorly. The muscularis mucosae was absent as far back as the caudal part of the pharynx. Uniformly constructed salivary glands were observed in groups in the submucosa.

Large mucous glands were present in the tunica propria of the esophagus. No glands were found in the diverticulum of the crop but were confined to the esophageal wall of that organ.

The stratified squamous epithelium of the esophagus changed into simple columnar epithelium at the junction with the proventriculus and continued as such as far as the anus.

The proventriculus contained simple tubular-formed superficial pro-pria glands and multilobular deep pro-pria glands.

Between the proventriculus and the gizzard was an intermediary zone characterized by the disappearance of the deep pro-pria glands and a lengthening of the superficial glands with the appearance of a keratinized inner layer.

The gizzard was characterized by a keratinized inner layer. Tubular glands, which emptied on the surface, were arranged in groups in the tunica pro-pria. The lamina muscularis was a single layer of involuntary muscle.

The intestine from the gizzard to the anus, including the caeca, was characterized by villi. No Brunner's glands were present in the duodenum. No Peyer's patches were found.

The caeca contained many plica circulares. The villi were low to absent in the blind end.

The cloaca contained many transverse folds and plica circulares in its walls.

The anus was lined with stratified squamous epithelium, and contained voluntary muscle in its walls.

The liver and the pancreas were similar in structure to those of mammals. The two pancreatic and the three bile ducts opened into the duodenal papilla.

The bursa cloacae was an organ of lymphoid-epithelial structure found only in chickens under one year of age.

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PLATE I

Entire digestive tract of a baby chick.

The duodenum was separated from the pancreas and the loop opened.
The pancreas and liver were joined to the intestine by the ducts.

- | | |
|---------------------------|---|
| 1. Beak | 13. Pancreas |
| 2. Tongue | 14. Gall bladder |
| 3. Mouth | 15. Liver |
| 4. Pharynx | 16. Jejunum and ileum |
| 5. Esophagus | 17. Yolk sac |
| 6. Crop | 18. Yolk stalk |
| 7. Proventriculus | 19. Caeca |
| 8. Ventriculus or gizzard | 20. Junction of caeca and small intestine |
| 9. Mm. laterales | 21. Rectum |
| 10. Mm. intermedii | 22. Cloaca |
| 11. Tendinous aponeurosis | 23. Bursa cloacae |
| 12. Duodenum | 24. Anus |

Plate I

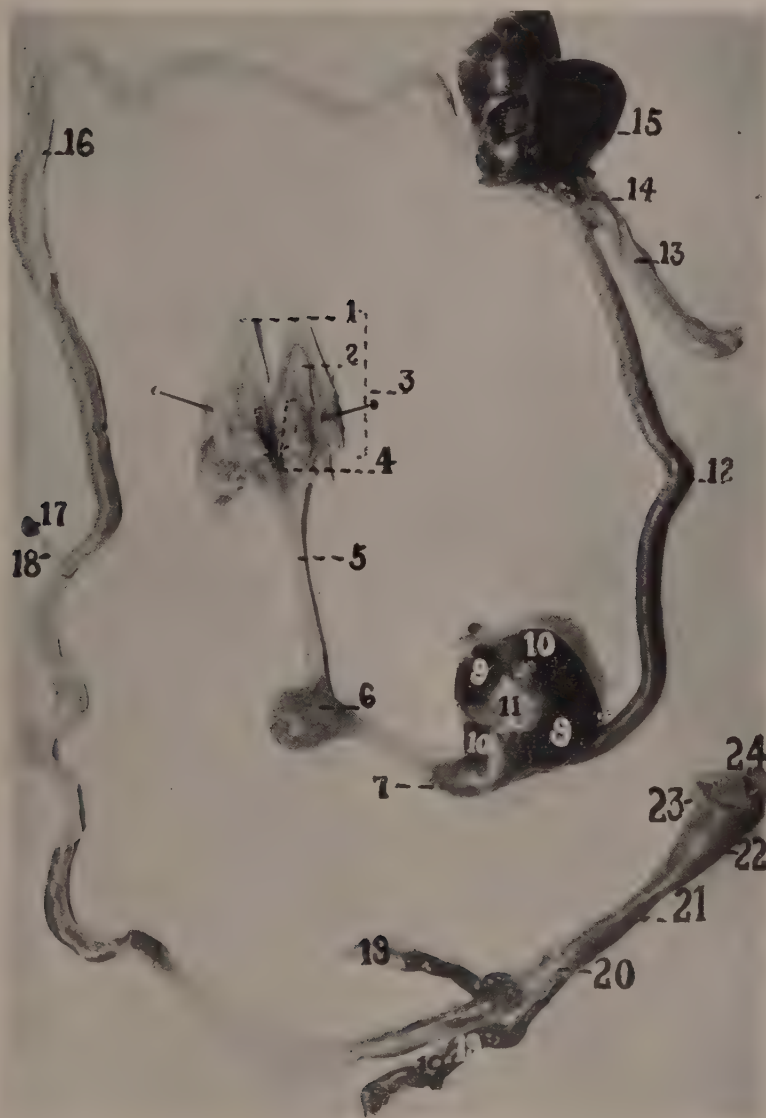


PLATE II

Mouth parts of the chicken.

1. Opening of the maxillary gland
2. Openings of the lateral palatine gland
3. Openings of the medial palatine gland
4. Nasal opening
5. Papillae separating mouth from pharynx
6. Pharynx
7. Openings of the sphenopterygoid gland
8. Opening of the Eustachian tubes
9. Pharyngeal papillae
10. Aditus esophagus with mucous gland opening on its surface
11. Esophagus
12. Tongue
13. Lingual papillae
14. Openings of the posterior lingual gland
15. Openings from the three parts of the posterior submaxillary gland
16. Aditus laryngis
17. Openings of the cricoarytenoid gland
18. Laryngeal papillae

Plate II

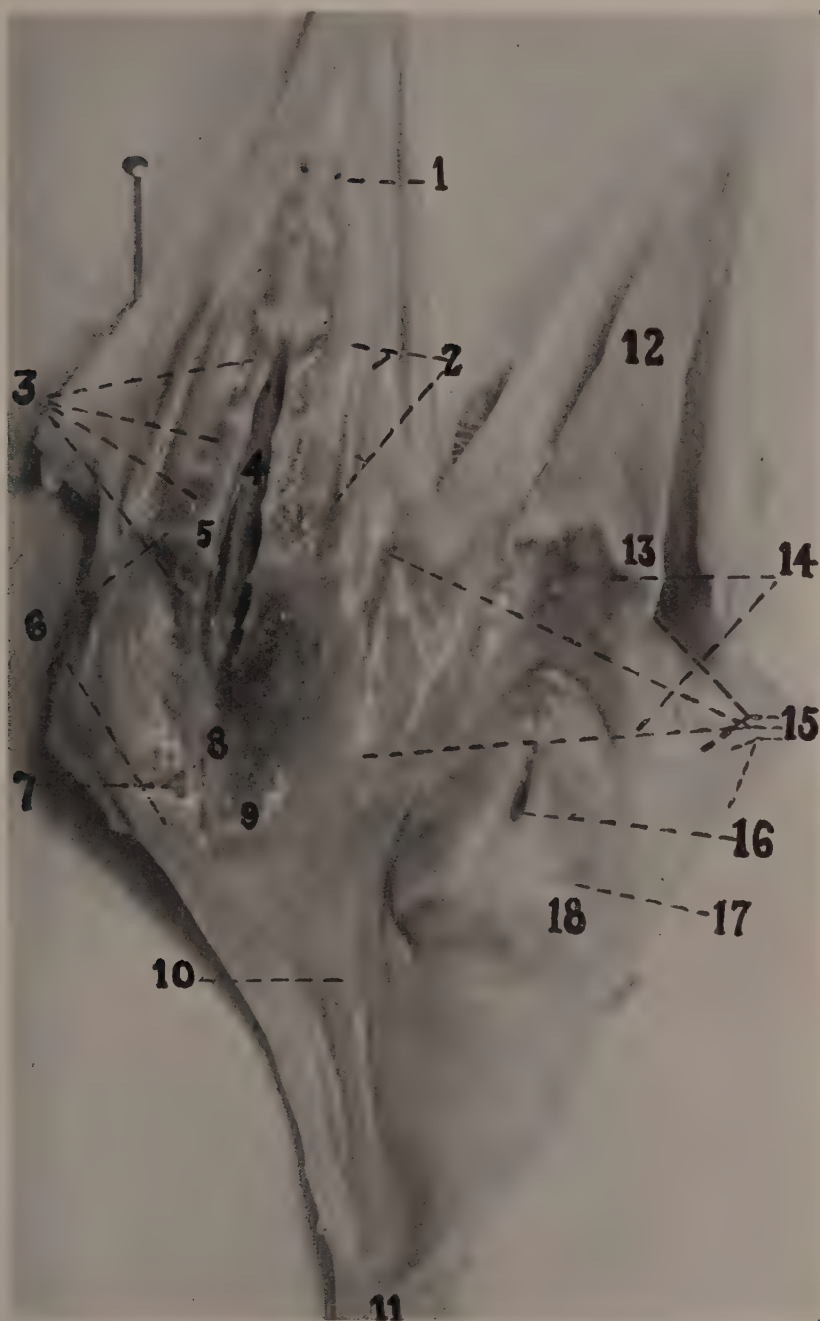


PLATE III

Photograph of a drawing to show the pancreas, the pancreatic ducts, and the bile ducts.

1. Gizzard
2. Proventriculus
3. Spleen
4. Liver
5. Gall bladder
6. Ductus cysticus
7. Ductus hepaticus
8. Pancreatic ducts from the dorsal pancreas
9. Pancreatic ducts from the ventral pancreas
10. Ventral lobe of the pancreas
11. Dorsal lobe of the pancreas
12. Splenic lobe of the pancreas
13. Esophagus
14. Vena cava
15. Duodenum
16. Small intestine

Plate III

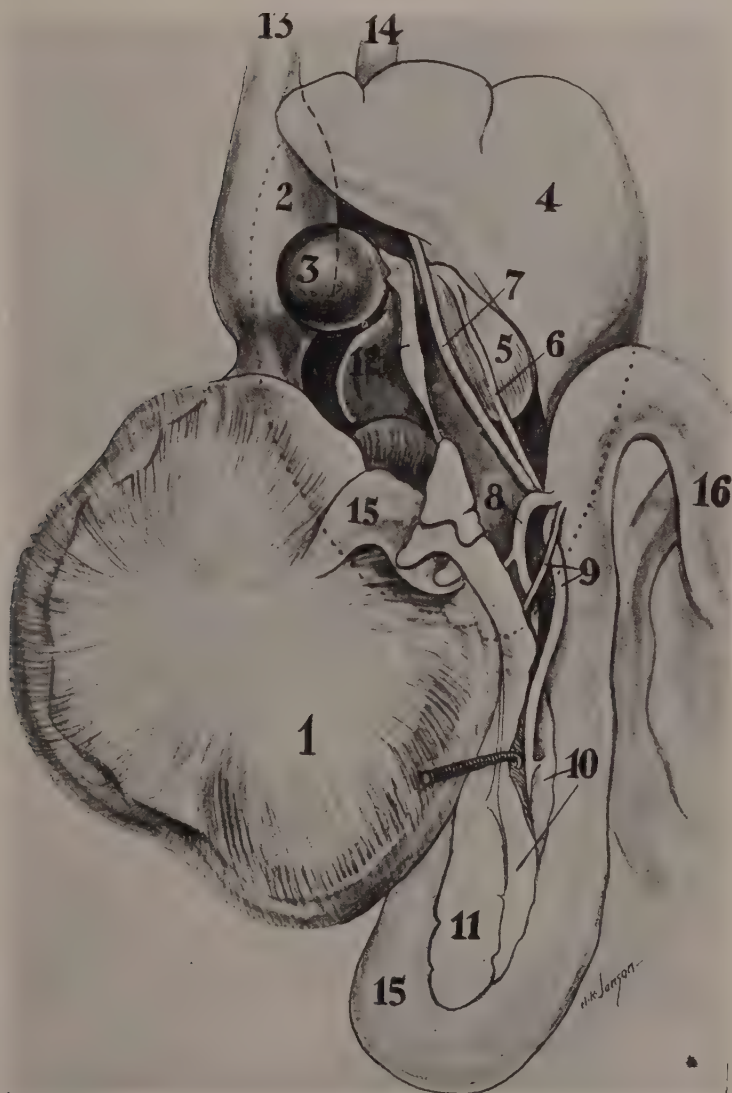


PLATE IV

Fig. A. Upper beak, cross section. Hematoxylin-eosin. 200 x. 45 days.

1. Epidermis
2. Stratum corneum
3. Stratum lucidum
4. Stratum granulosum
5. Stratum germinativum
6. Corium
7. Blood vessels
8. Nerve
9. Taste corpuscle
10. Periosteum
11. Bone

Fig. B. Lower beak, longitudinal section. Hematoxylin-eosin. 200 x. 45 days.

1. Epidermis
2. Stratum corneum
3. Stratum lucidum
4. Stratum granulosum
5. Stratum germinativum
6. Corium
7. Blood vessels
8. Periosteum
9. Bone
10. Submucosa
11. Tunica propria
12. Epithelium of mouth

Plate IV

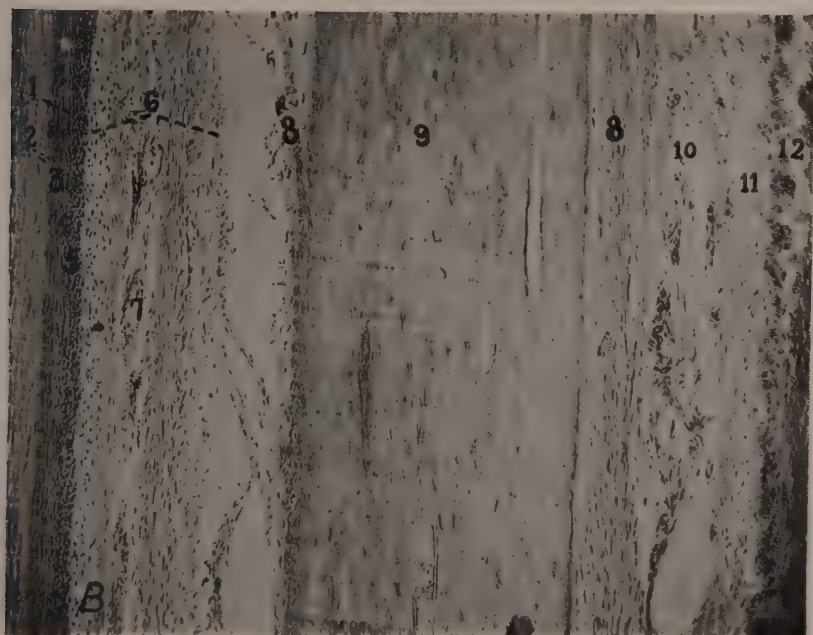


PLATE V

Fig. A. Anterior portion of the hard palate.

- | | |
|-------------------|-----------------------------|
| 1. Epithelium | 4. Fat |
| 2. Tunica propria | 5. Excretory duct |
| 3. Submucosa | 6. Maxillary salivary gland |

Fig. B. Mid-portion of the hard palate.

- | | |
|-------------------|-----------------------------------|
| 1. Epithelium | 4. Fat |
| 2. Tunica propria | 5. Medial palatine salivary gland |
| 3. Submucosa | 6. Papilla of hard palate |

Fig. C. Region adjacent to the nares.

- | | |
|-------------------|-----------------------------------|
| 1. Epithelium | 4. Nasal mucous membrane |
| 2. Tunica propria | 5. Medial palatine salivary gland |
| 3. Submucosa | 6. Papilla on hard palate |

Plates V-VII represent a longitudinal section through the roof of the mouth and pharynx. The figures are a series from a section extending from the beak to the esophagus. Hematoxylin-eosin. 50 x. 2 weeks.

Plate V

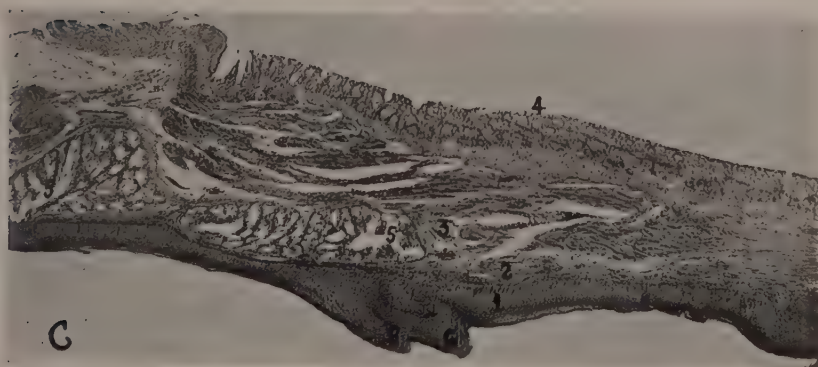
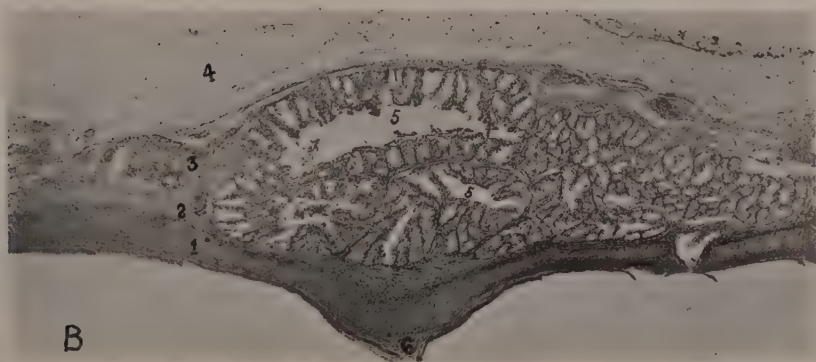
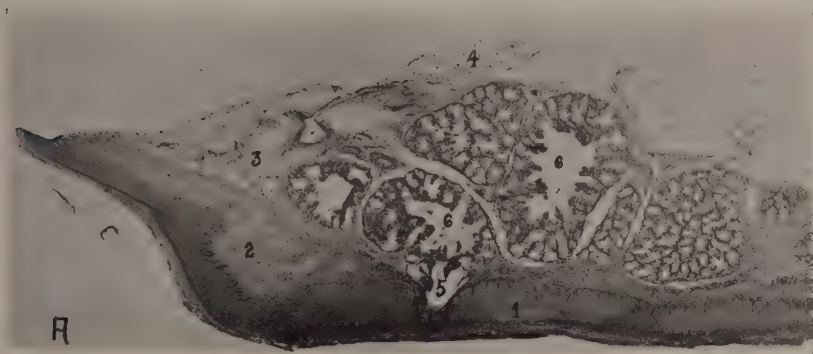


PLATE VI

Fig. A. A longitudinal section through the mucosa lining the nares.

1. Epithelium
2. Tunica propria
3. Papillae separating the roof of the mouth from the pharynx

Fig. B. Anterior portion of the pharynx

- | | |
|-------------------|--|
| 1. Epithelium | 4. Epithelium of nasal cavity |
| 2. Tunica propria | 5. Medial palatine gland |
| 3. Submucosa | 6. Papillae of the roof of the pharynx |

Fig. C. Mid-portion of the pharynx.

- | | |
|---|--|
| 1. Epithelium | 4. Medial palatine gland |
| 2. Tunica propria | 5. Sphenopterygoid salivary gland |
| 3. Muscle arranged obliquely to the roof of the pharynx | 6. Papillae of the roof of the pharynx |

Plate VI

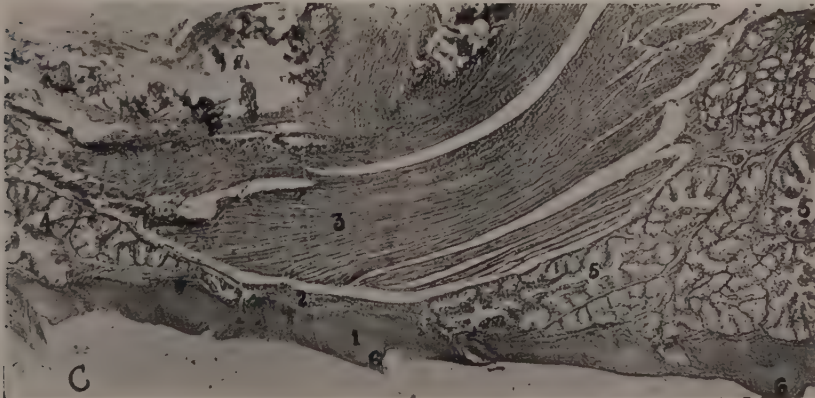
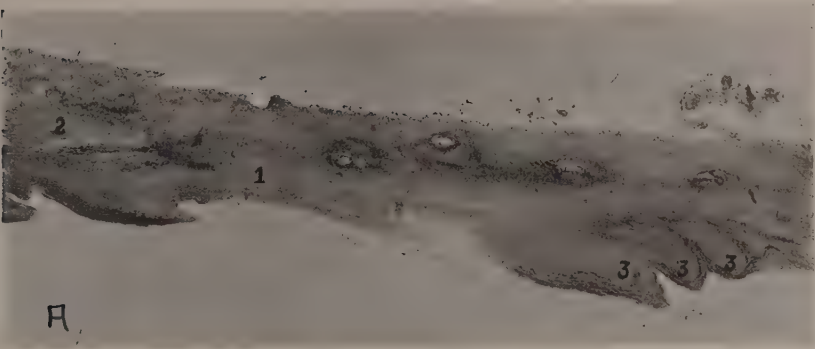


PLATE VII

Fig. A. Region from pharynx to esophagus.

1. Epithelium
2. Tunica propria
3. Muscularis mucosae
4. Submucosa
5. Beginning lamina muscularis
6. Adventitia
7. Sphenopterygoid salivary gland
8. Papilla separating the pharynx from the esophagus

Fig. B. Esophagus.

1. Epithelium
2. Tunica propria with mucous glands
3. Muscularis mucosae
4. Submucosa
5. Circular layer of lamina muscularis
6. Longitudinal layer of lamina muscularis
7. Adventitia

Plate VII

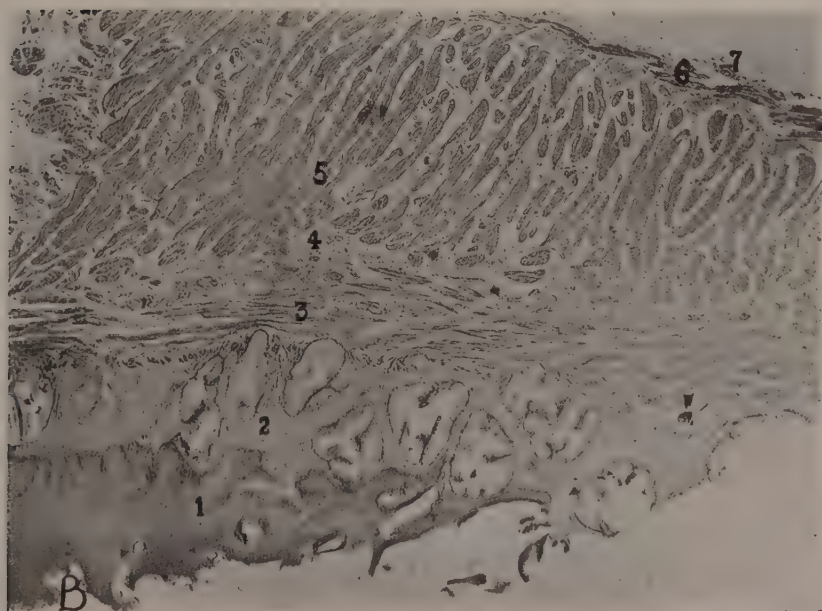
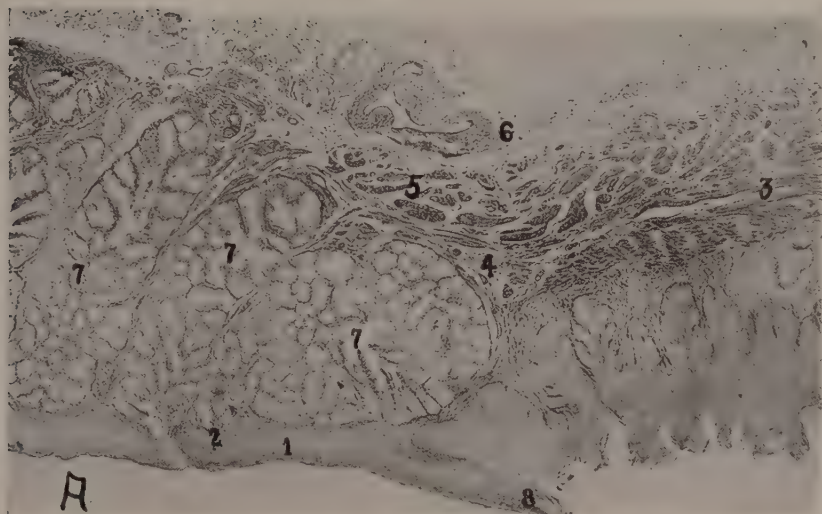


PLATE VIII

Fig. A. Cross section of tongue near tip. Hematoxylin-eosin. 50 x. Baby chick.

1. Epithelium of dorsal surface
2. Tunica propria
3. Anterior lingual salivary glands
4. Cartilage (entoglossal bone)
5. Muscle
6. Epithelium of ventral surface showing cornification

Fig. B. Cross section of tongue, mid portion. Hematoxylin-eosin. 50 x. one and one-half years.

1. Epithelium of dorsal surface
2. Tunica propria
3. Anterior lingual salivary glands
4. Entoglossal bone
5. Muscle
6. Epithelium of ventral surface

Plate VIII

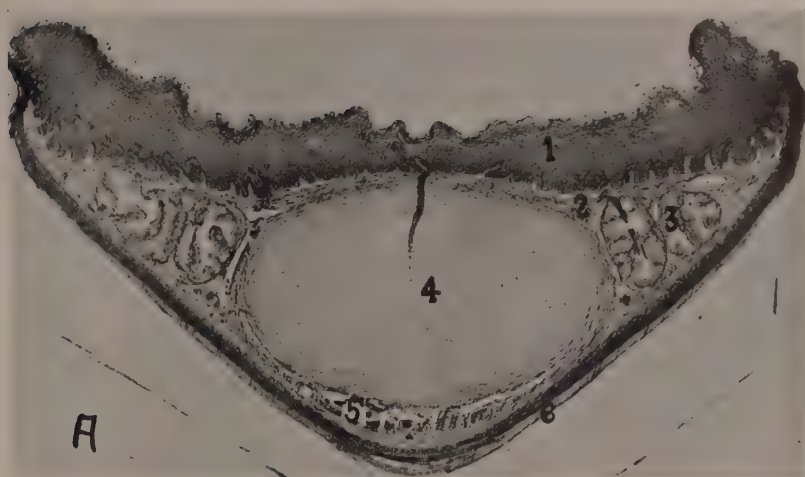


PLATE IX

Fig. A. Base of tongue. Hematoxylin-eosin. 25 x. Baby chick.

1. Epithelium
2. Papillae
3. Tunica propria
4. Submucosa
5. Cartilage (basihyal bone)
6. Cartilage (entoglossal bone)
7. Muscle
8. Lingual glands

Fig. B. Cross section of Aditus laryngis. Hematixylin-eosin. 50 x.
one and one-half years.

1. Epithelium
2. Lymphoid tissue in tunica propria
3. Lymphoid nodule
4. Submucosa
5. Cricoarytenoid gland

Plate IX

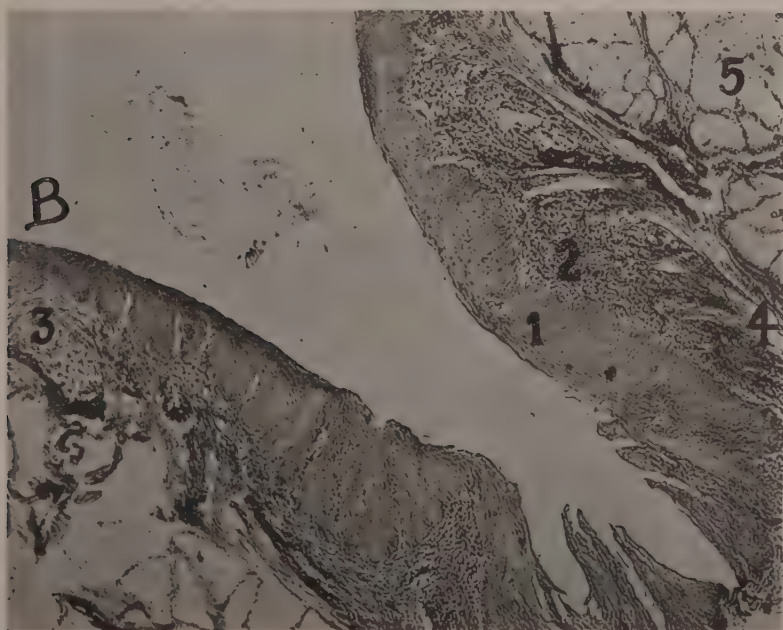


PLATE X

Fig. A. Posterior lingual salivary gland (without lymphoid tissue). Hematoxylin-eosin. 200 x. 20 days.

1. Duct with secretion pouring out
2. Epithelium of floor of pharynx
3. Central collecting cavity
4. Simple tubular gland
5. Gland capsule

Fig. B. Sphenopterygoid salivary gland. Hematoxylin-eosin. 200 x. 20 days.

1. Excretory duct
2. Epithelium of roof of pharynx
3. Tunica propria
4. Submucosa
5. Cross section of a gland tubule
6. Gland capsule

Plate X

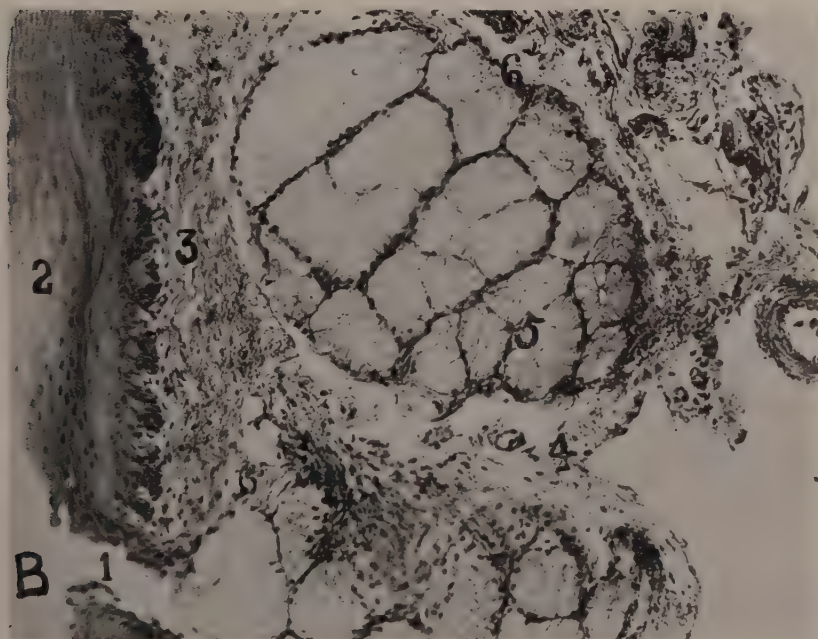
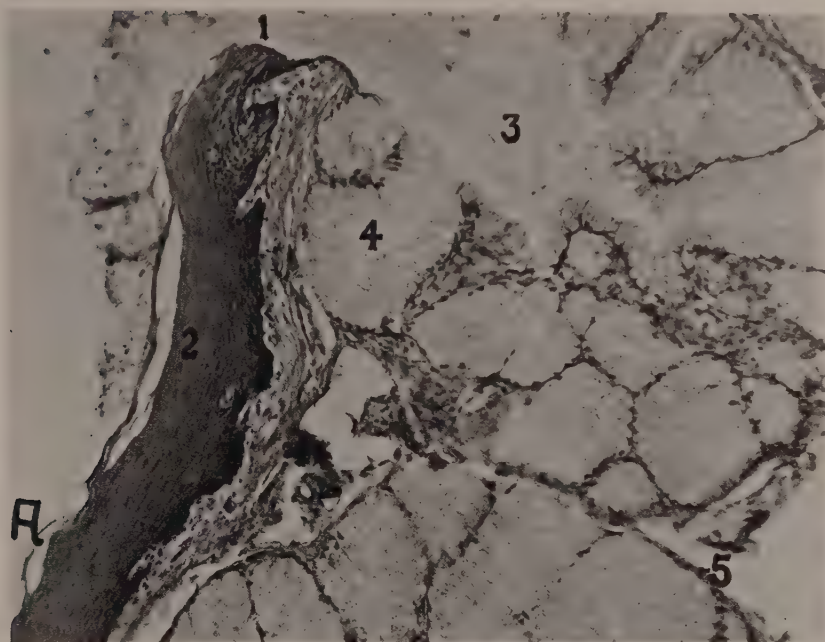


PLATE XI

Fig. A. Sphenopterygoid salivary gland with lymphoid tissue. Hematoxylin-eosin.
200 x. 2 years.

1. Epithelium of pharynx
2. Tunica propria
3. Submucosa
4. Gland showing infiltration with lymphoid tissue
5. Glandular area with cell outlines absent

Fig. B. Posterior submaxillary salivary gland stained for mucin. 200 x. 5 months.

1. Submucosa
2. Posterior submaxillary salivary gland

Plate XI

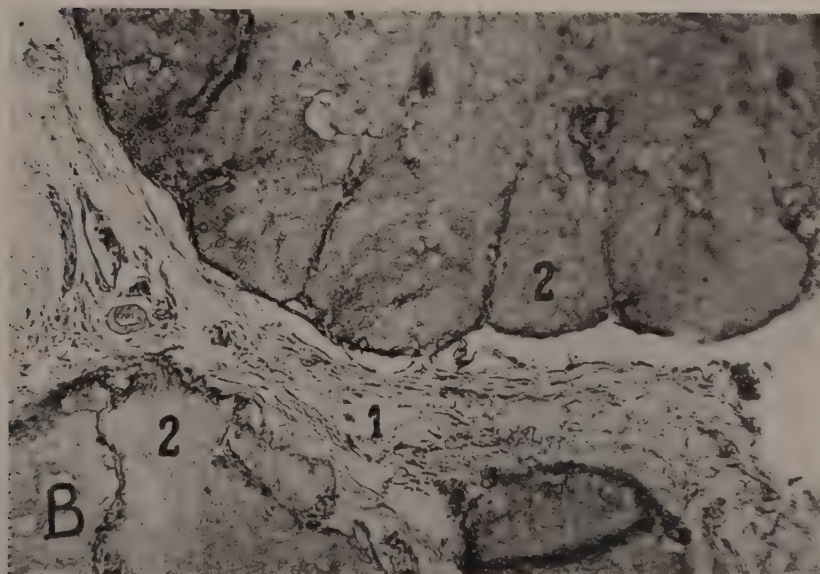
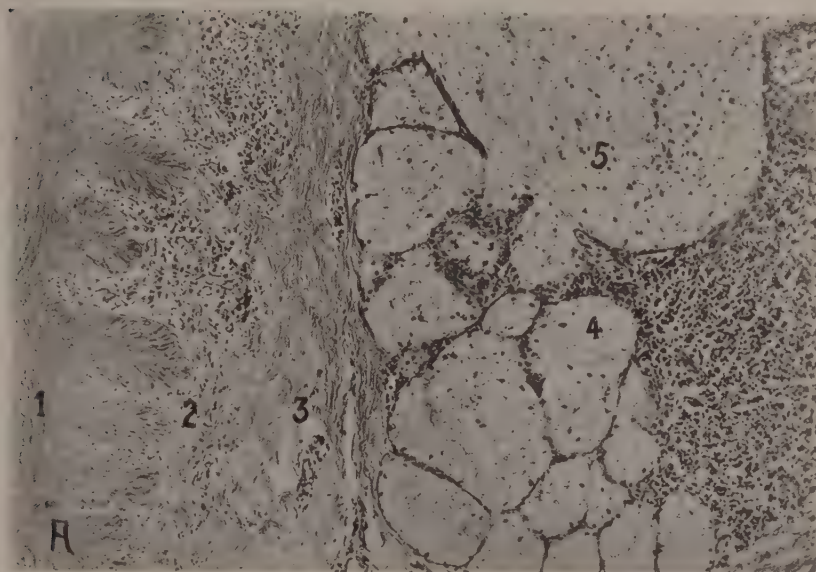


PLATE XII

Fig. A. Maxillary salivary gland. Cross section from anterior portion of roof of mouth. Hematoxylin-eosin. 50 x. 5 days.

1. Papilla of roof of mouth
2. Epithelium
3. Tunica propria
4. Muscle
5. Submucosa
6. Gland tissue

Fig. B. Lobule of submaxillary salivary gland. Mucous stain. 50 x. 2 years.

1. Epithelium of roof of mouth
2. Excretory duct
3. Tunica propria
4. Submucosa
5. Gland tissue showing different densities of mucin
6. Lymphoid tissue
7. Gland capsule

Plate XII

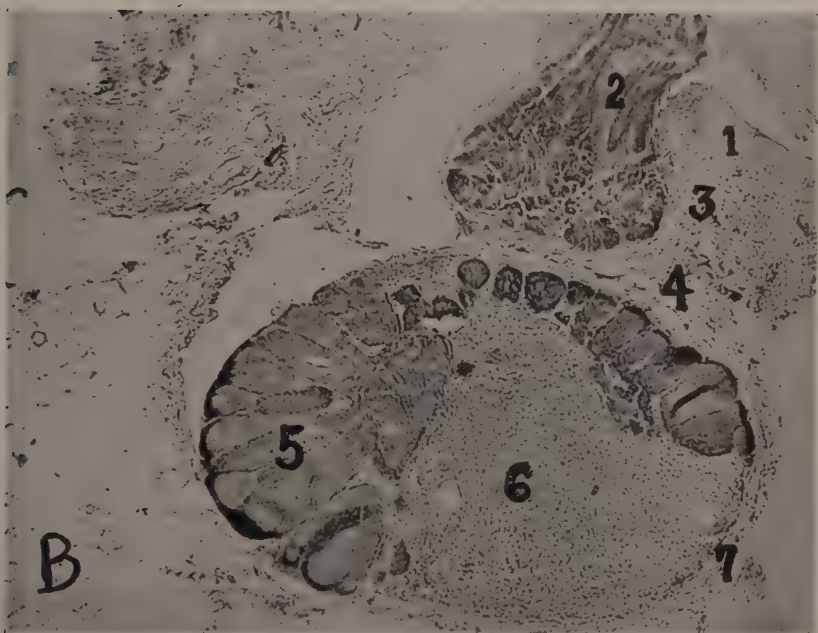
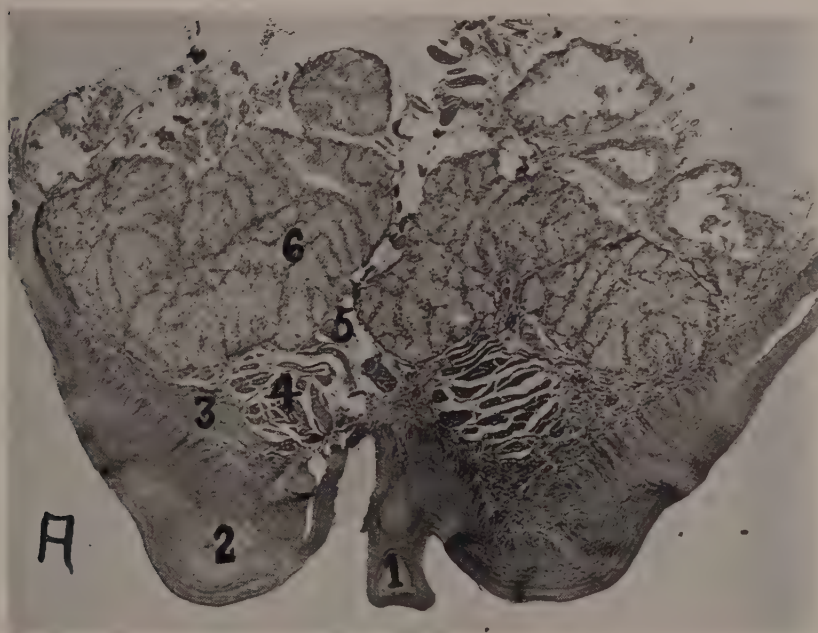


PLATE XIII

Fig. A. Esophagus (near crop) cross section. Hematoxylin-eosin. 50 x. 20 days.

1. Epithelium
2. Tunica propria
3. Mucous glands
4. Muscularis mucosae
5. Submucosa
6. Inner circular layer of the lamina muscularis
7. Outer longitudinal layer of the lamina muscularis
8. Adventitia

Fig. B. Esophagus (same as above). Hematoxylin-eosin. 200 x. 30 days.

1. Epithelium
2. Tunica propria
3. Mucous glands
4. Muscularis mucosae
5. Submucosa
6. Blood vessel
7. Circular layer of lamina muscularis

Plate XIII

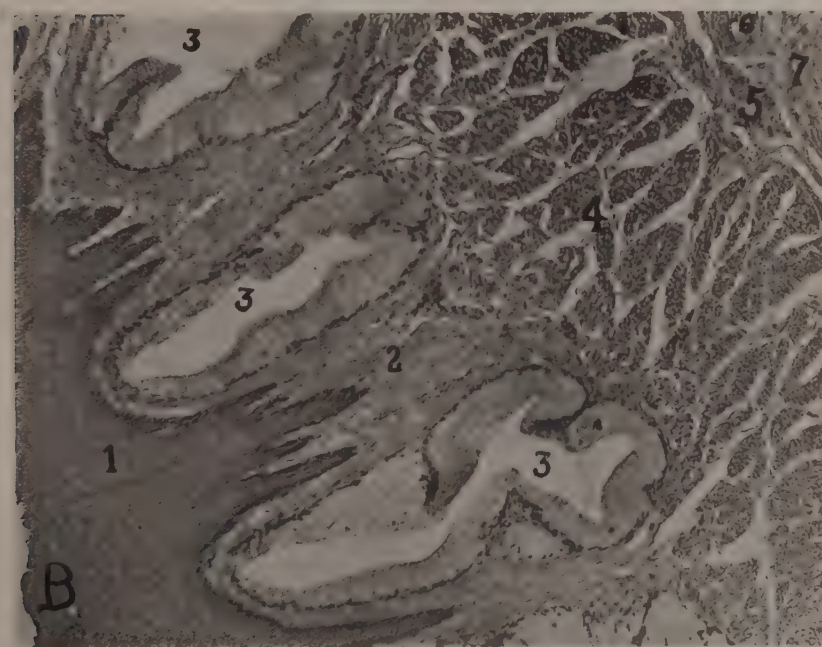
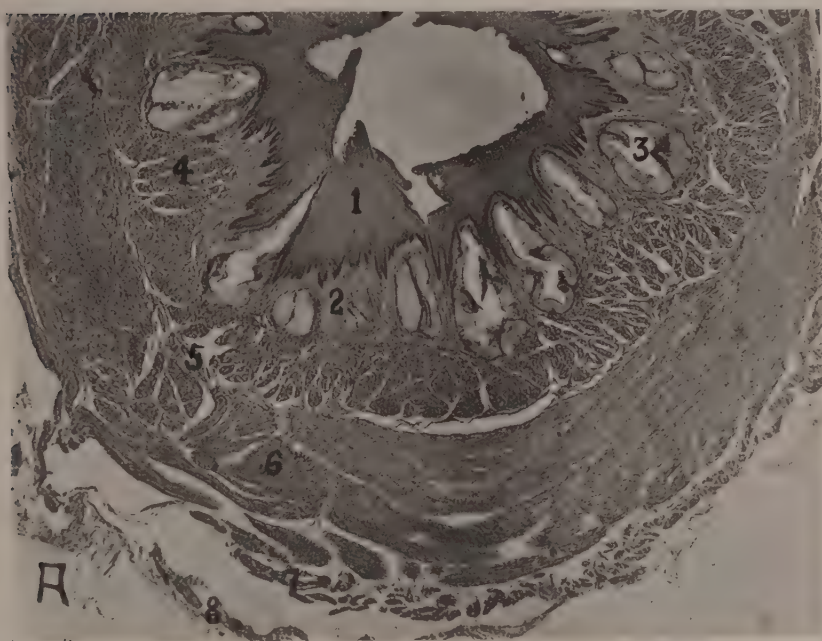


PLATE XIV

Fig. A. Esophagus showing leucocytic infiltration of the glands. Midportion, cross section. Hematoxylin-eosin. 50 x. One and one-half years.

1. Epithelium
2. Tunica propria
3. Muscularis mucosae
4. Mucous gland with lymphoid tissue
5. Submucosa
6. Circular muscle layer of lamina muscularis.

Fig. B. Mucous gland opening into lumen of esophagus. Hematoxylin-eosin. 200 x. One and one-half years.

1. Epithelium
2. Tunica propria
3. Excretory duct
4. Gland with lymphoid tissue
5. Collecting cavity
6. Gland tubules

Plate XIV

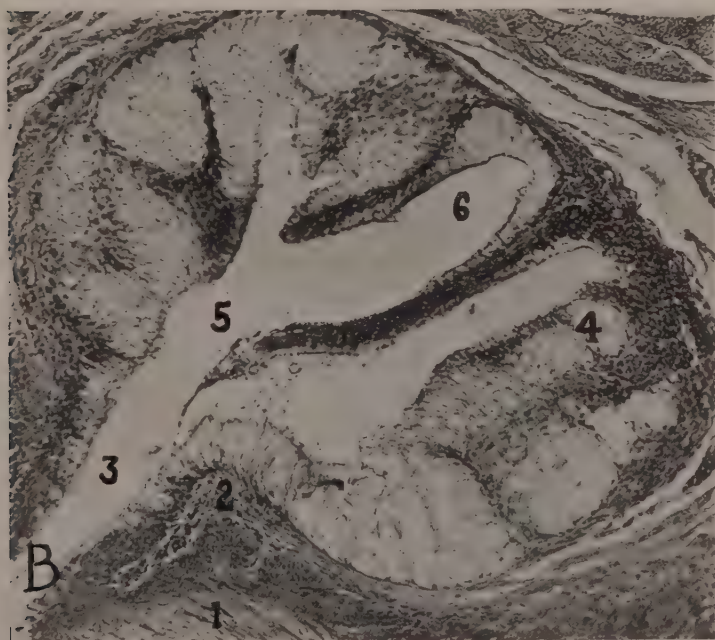


PLATE XV

Fig. A. Elastic tissue (fine black lines) in the tunica propria of the esophagus. Weigert's. 200 x. 20 days.

1. Epithelium
2. Tunica propria
3. Muscularis mucosae

Fig. B. Elastic tissue in the sub-mucosa of the esophagus. (Black in photograph). Weigert's. 200 x. 20 days.

1. Mucous gland
2. Muscularis mucosae
3. Submucosa (elastic tissue intense black)
4. Circular layer of lamina muscularis

Plate XV

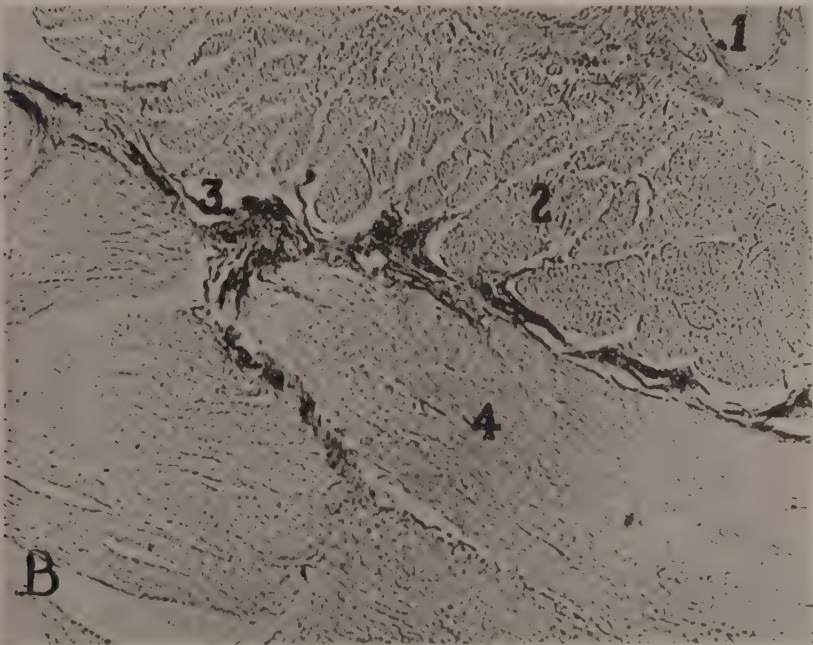
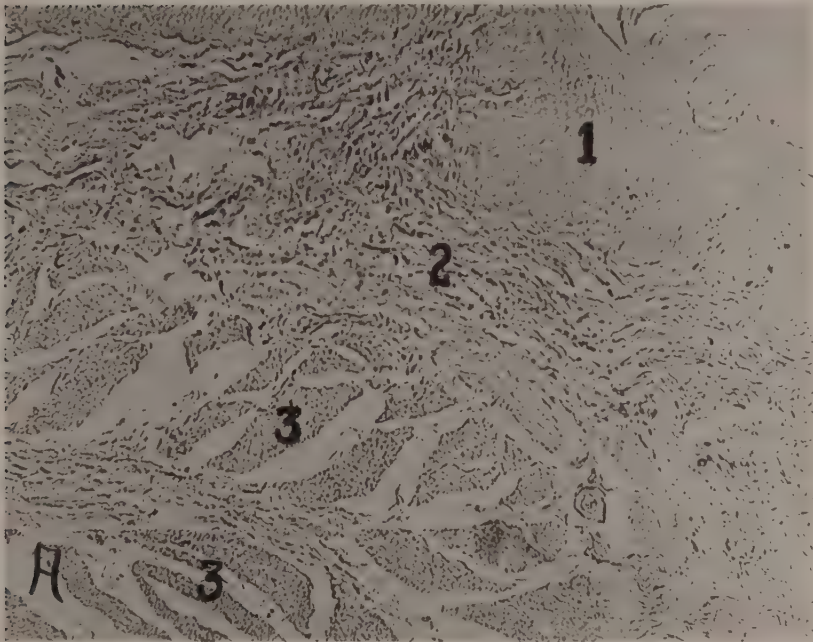


PLATE XVI

Fig. A. Crop (diverticulum), cross section. Hematoxylin-eosin. 200 x. 36 hours.

1. Epithelium
2. Tunica propria
3. Muscularis mucosae
4. Submucosa
5. Circular layer of the lamina muscularis
6. Longitudinal layer of the lamina muscularis
7. Adventitia

Fig. B. Crop (esophageal wall), cross section. Hematoxylin-eosin. 50 x. 20 days.

1. Epithelium
2. Tunica propria
3. Mucous glands
4. Muscularis mucosae
5. Submucosa
6. Circular layer of the lamina muscularis
7. Longitudinal layer of the lamina muscularis
8. Adventitia (with many blood vessels)

Plate XVI

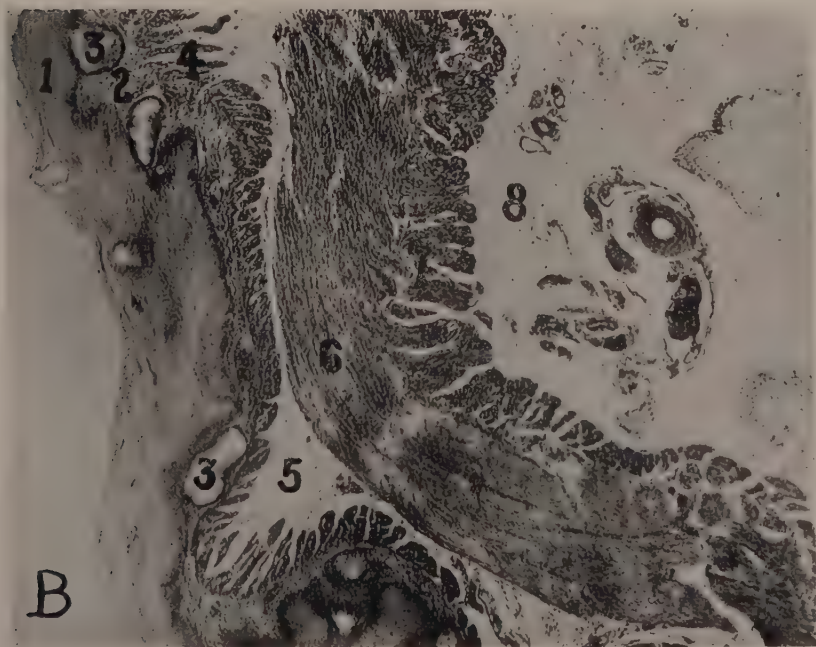
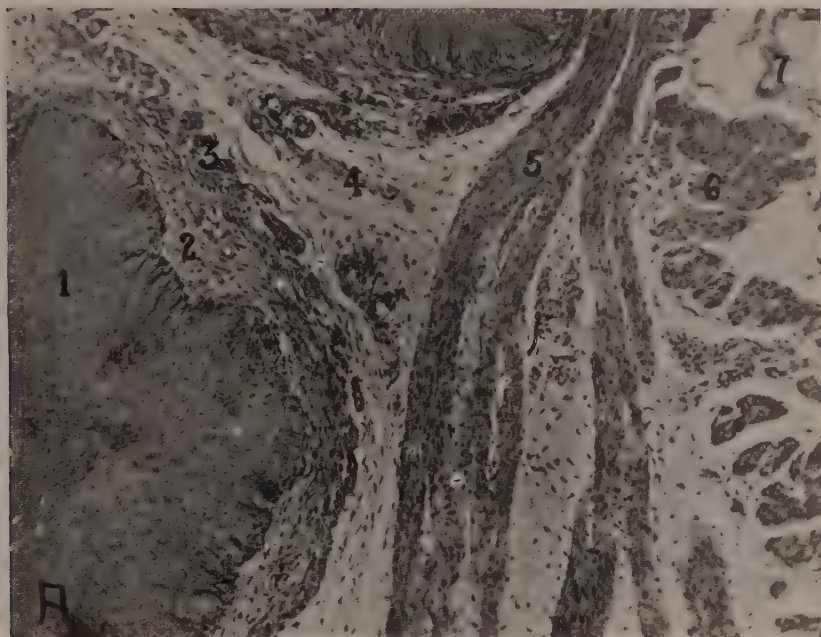


PLATE XVII

Fig. A. Proventriculus showing opening of deep gland on the surface. (Organ inflated before fixation). Cross section. Hematoxylin-eosin. 50 x. Age unknown (adult).

1. Epithelium
2. Tubular glands of the surface
3. Tunica propria
4. Opening of a deep gland on the surface
5. Collecting cavity
6. Gland lobule
7. Muscularis mucosae
8. Junction of muscularis mucosae and lamina muscularis (submucosa)
9. Circular layer of lamina muscularis
10. Longitudinal layer of lamina muscularis
11. Adventitia

Fig. B. Proventriculus (not inflated). Cross section. Hematoxylin-eosin. 50 x. 36 hours.

1. Surface tubular glands lined with columnar epithelium
2. Tunica propria
3. Gland lobule
4. Muscularis mucosae
5. Circular layer of lamina muscularis
6. Longitudinal layer of lamina muscularis
7. Adventitia

Plate XVII

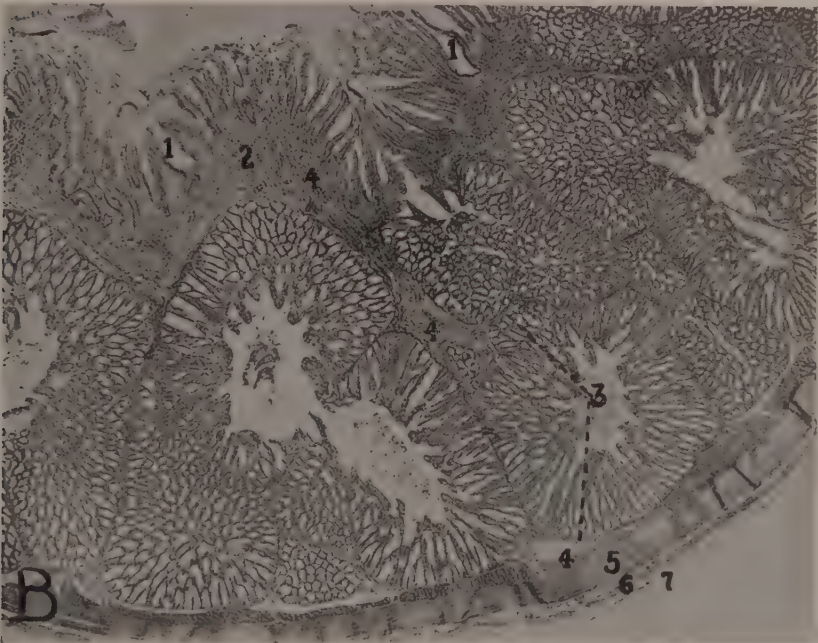


PLATE XVIII

Fig. A. Proventriculus. Hematoxylin-eosin. 200 x. 36 hours.

1. Cross sections of gland tubules
2. Longitudinal sections of gland tubules
3. Septa between lobules
4. Gland cells
5. A mass of blood cells in a blood vessel

Fig. B. Elastic tissue in area between proventriculus and gizzard. (Elastic tissue black). Weigert's 200 x. 2 years.

1. Gland layer
2. Tunica propria
3. Muscularis mucosae

Plate XVIII

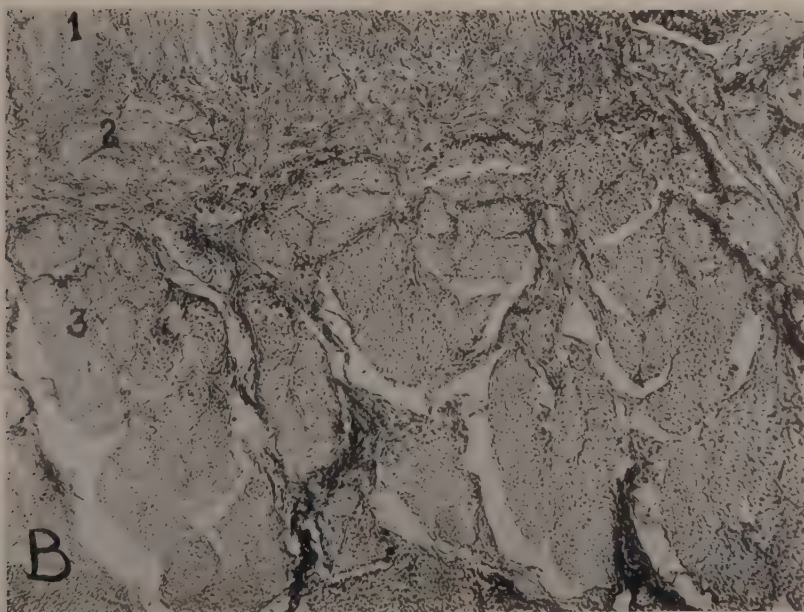
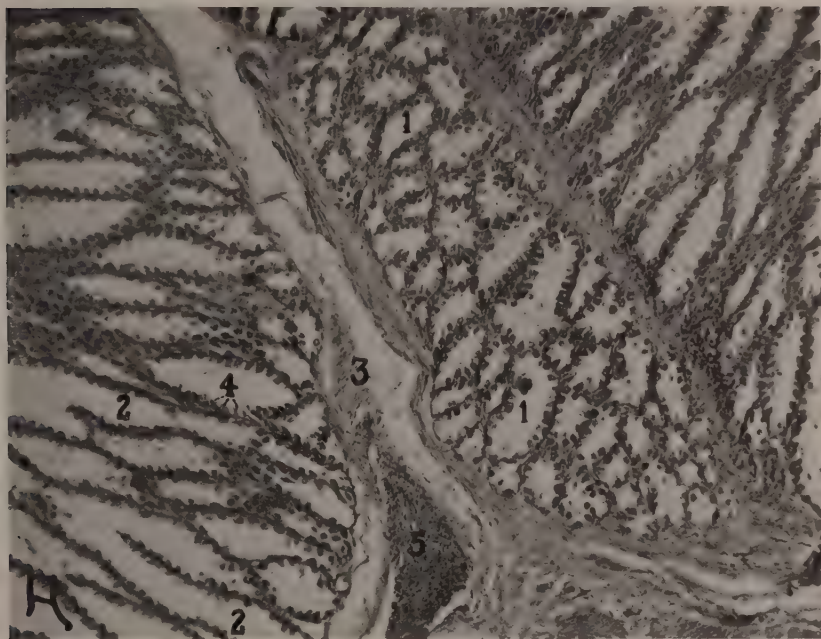


PLATE XIX

Fig. A. Entire wall of gizzard. Hematoxylin-eosin. 50 x. 3 days.

1. Horny layer
2. Glands in the tunica propria
3. Submucosa
4. Muscle
5. Serosa

Fig. B. Gizzard showing horny layer in detail. Keratohyalin stain. 200 x. 5 months.

1. Horny layer
2. Wavy line parallel to the surface
3. Lines perpendicular to the surface
4. Exudate in gland tubules
5. Glandular layer

Plate XIX

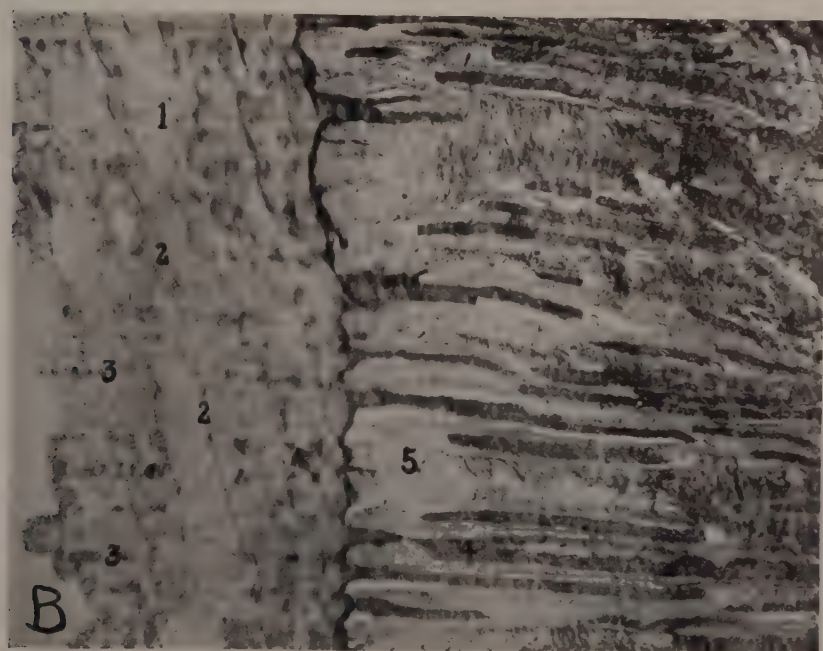


PLATE XX

Fig. A. Gizzard showing keratohyalin stained in glandular layer. Keratohyalin stain. 200 x. 5 months.

1. Horny layer
2. Exudate in tubule
3. Gland tubule

Fig. B. Gizzard showing keratohyalin granules. Keratohyalin stain. 800 x. 36 hours.

1. Exudate in tubules
2. Tubule
3. Keratohyalin granules (fine black dots)

Plate XX

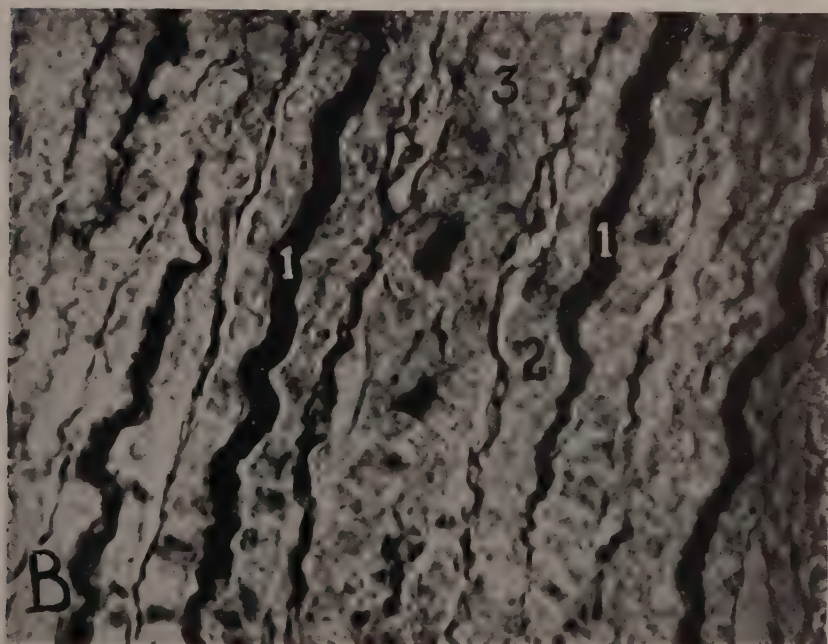


PLATE XXI

Fig. A. Gizzard. Hematoxylin-eosin. 200 x. 2 years.

1. Gland tubules in tunica propria. Note arrangements in groups
2. Septa of tunica propria
3. Muscle

Fig. B. Gizzard. Hematoxylin-eosin. 800 x. 2 years.

1. Tunica propria septa
2. Group of gland tubules
3. Flat cells with bulging nuclei
4. Lumen of a tubule

Plate XXI

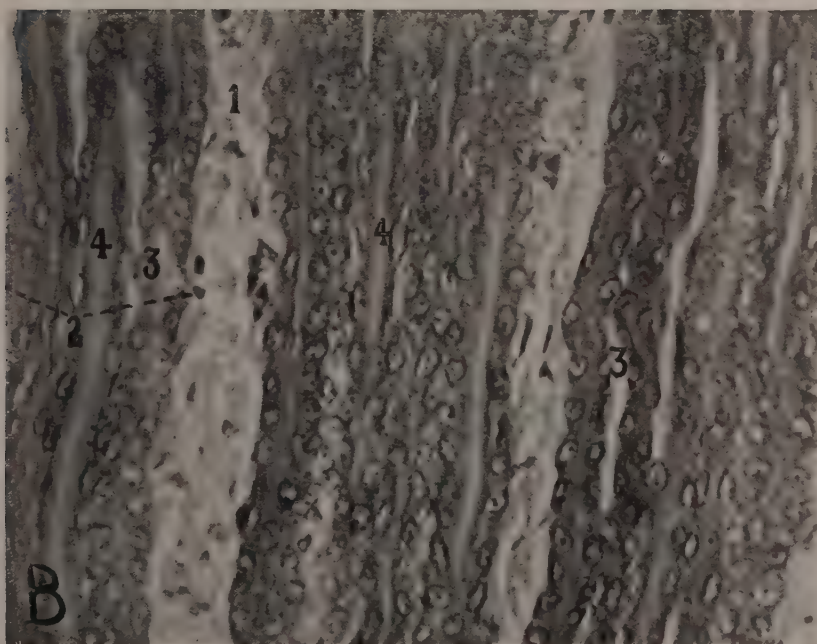
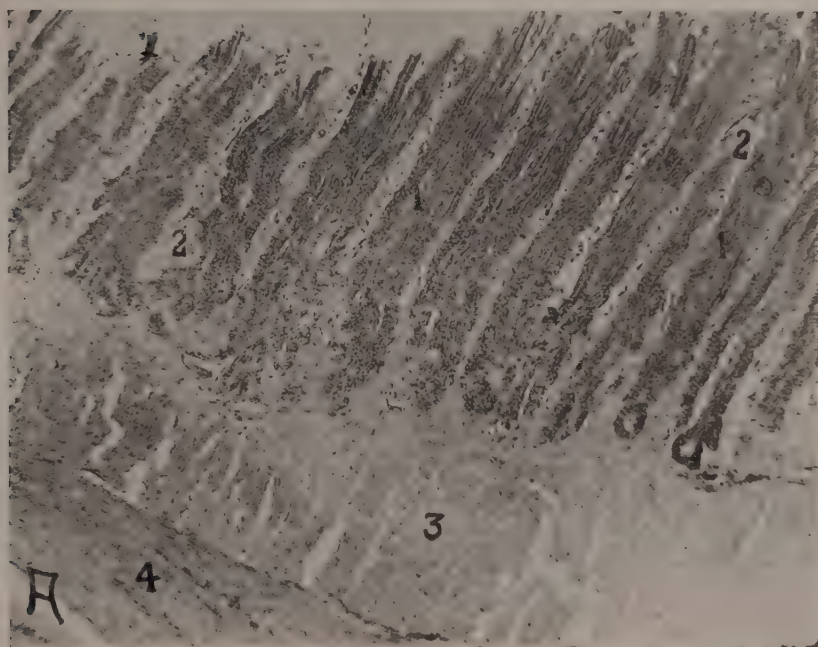


PLATE XXII

Fig. A. Types of duodenal villi. Hematoxylin-eosin. 200 x. 36 hours.

Fig. B. Lymphoid tissue in small intestine. Hematoxylin-eosin. 50 x. 2 years.

1. Longitudinal muscle
2. Circular muscle
3. Submucosa
4. Muscularis mucosae
5. Lymph nodules
6. Diffuse lymphoid tissue
7. Crypts of Lieberkühn

Plate XXII

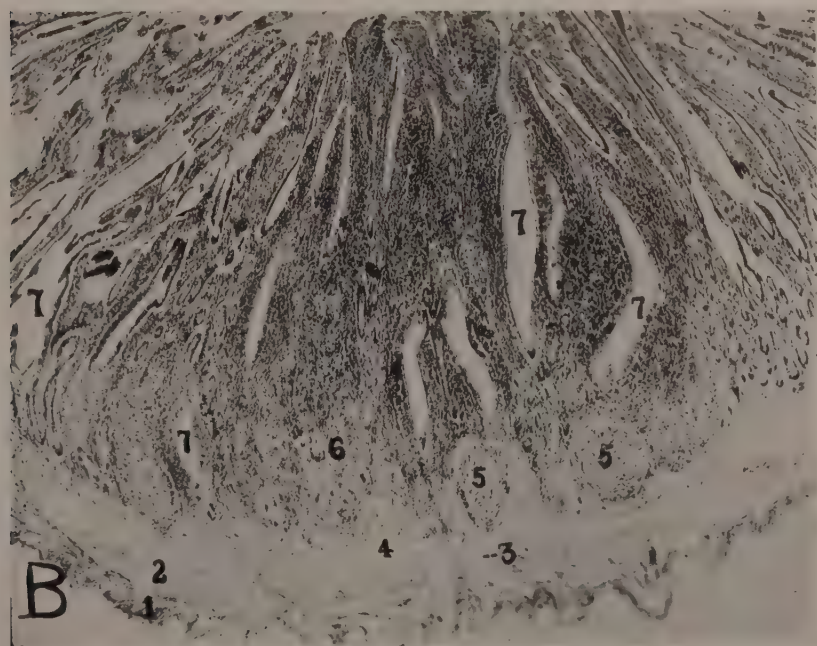
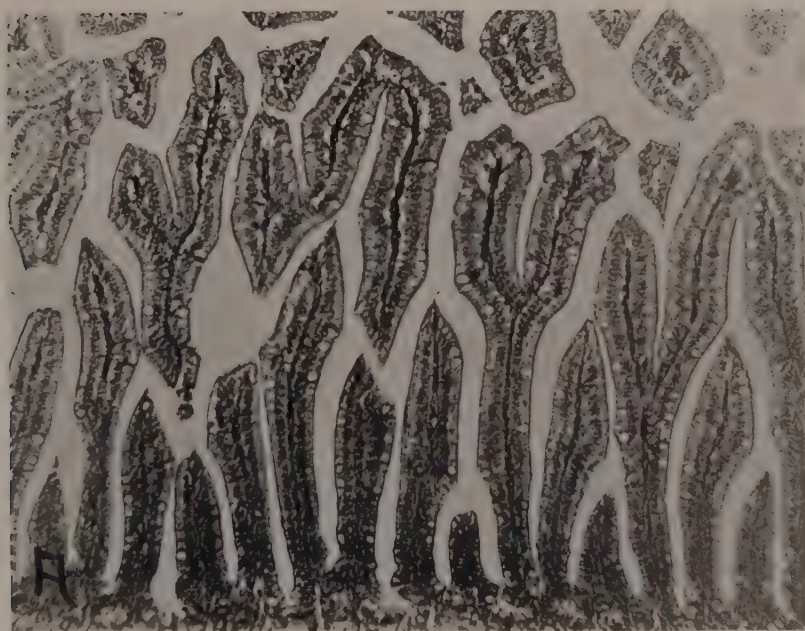


PLATE XXIII

Fig. A. Fibrous tissue in duodenum. Van Gieson's. 200 x. 2 years.

1. Tunica propria with glands of Lieberkühn
2. Muscularis mucosae
3. Submucosa
4. Lamina muscularis
5. Serosa

Fig. B. Elastic tissue in duodenum. Weigert's. 200 x. 2 years.

1. Tunica propria with glands of Lieberkühn
2. Muscularis mucosae
3. Submucosa
4. Lamina muscularis
5. Serosa
6. Vessels crossing circular layer of the lamina muscularis

Plate XXIII

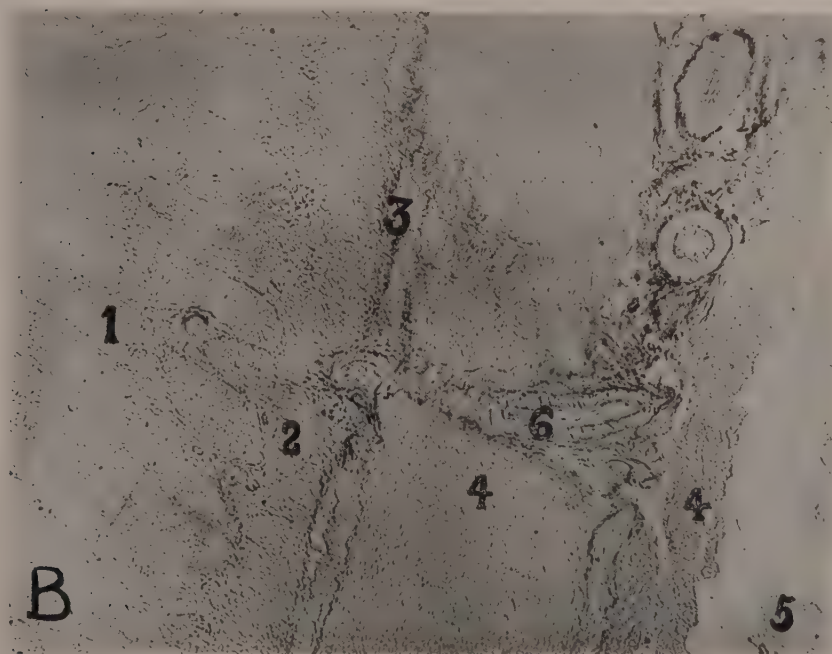


PLATE XXIV

Fig. A. Small intestine showing blood vessels entering the wall. Hematoxylin-eosin. 200 x. 2 years.

1. Vessels in adventitia
2. Longitudinal muscle layer
3. Circular muscle layer
4. Submucosa
5. Muscularis mucosae

Fig. B. Duodenum stained for mucous glands. Mucous stain. 200 x. One and one-half years.

1. Circular layer of lamina muscularis
2. Junction of muscularis mucosae and the circular layer of the lamina muscularis (submucosa)
3. Muscularis mucosae
4. Glands of Lieberkühn with goblet cells showing mucin.

Plate XXIV

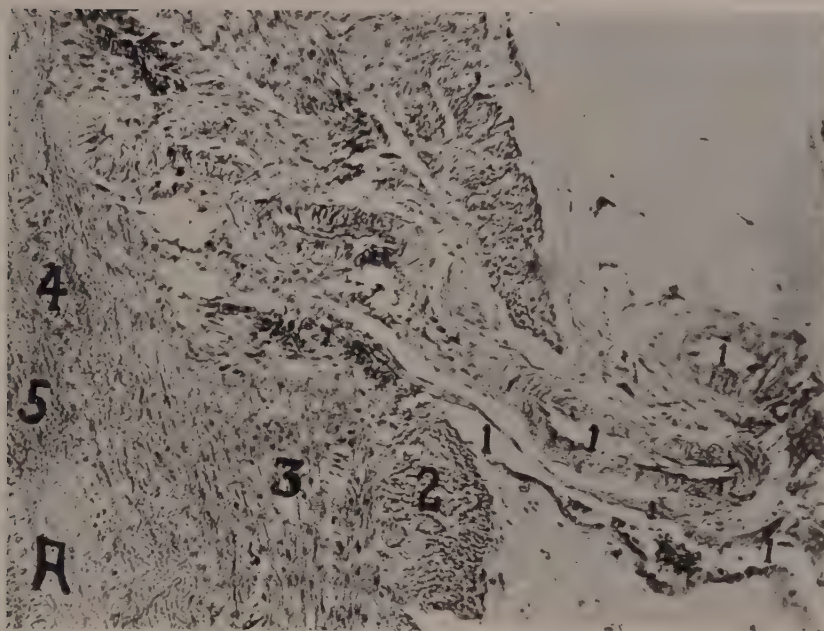


PLATE XXV

Fig. A. Longitudinal section of pancreatic and bile ducts entering the duodenum. Note the elevation in the mucous membrane of the duodenum. Hematoxylin-eosin. 50 x. One and one-half years.

1. Mucosa of the duodenum
2. Openings of ducts
3. Bile duct
4. Pancreatic ducts

Fig. B. Cross section of pancreatic and bile ducts near entrance into the duodenum. Hematoxylin-eosin. 50 x. 5 months.

1. Mucosa of the duodenum
2. Bile duct
3. Pancreatic ducts

Plate XXV

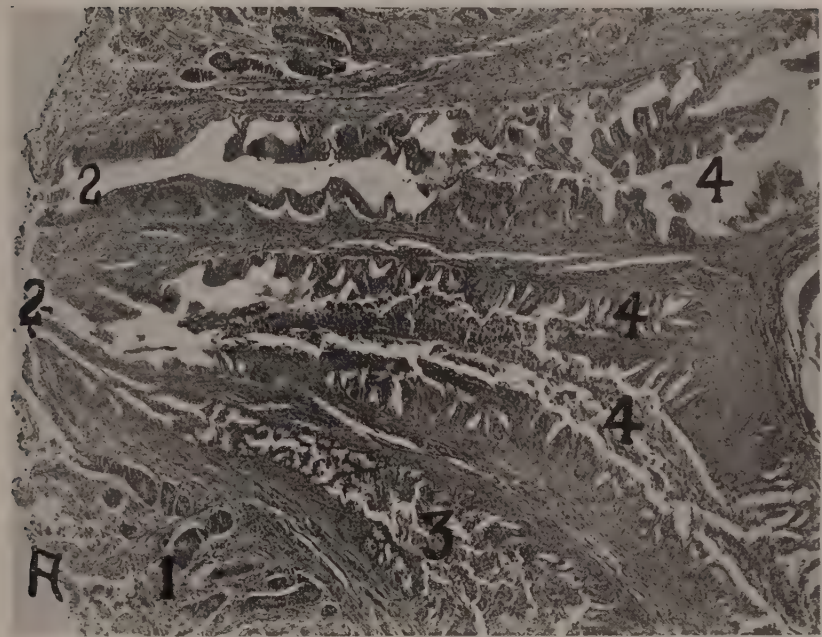


PLATE XXVI

Fig. A. Small intestine cross section. Hematoxylin-eosin. 200 x. 20 days.

1. Serosa
2. Longitudinal layer of the lamina muscularis
3. Circular layer of the lamina muscularis
4. Submucosa
5. Muscularis mucosae
6. Apparent fusion of outer circular layer of muscularis mucosa and inner circular layer of the lamina muscularis
7. Tunica propria
8. Glands of Lieberkühn
9. Crypts of Lieberkühn
10. Villi

Fig. B. Longitudinal section of the sphincter between the small intestine and rectum. Hematoxylin-eosin. 25 x. One and one-half years.

1. Mucosa of the small intestine
2. Mucosa of the rectum
3. Muscle

Plate XXVI

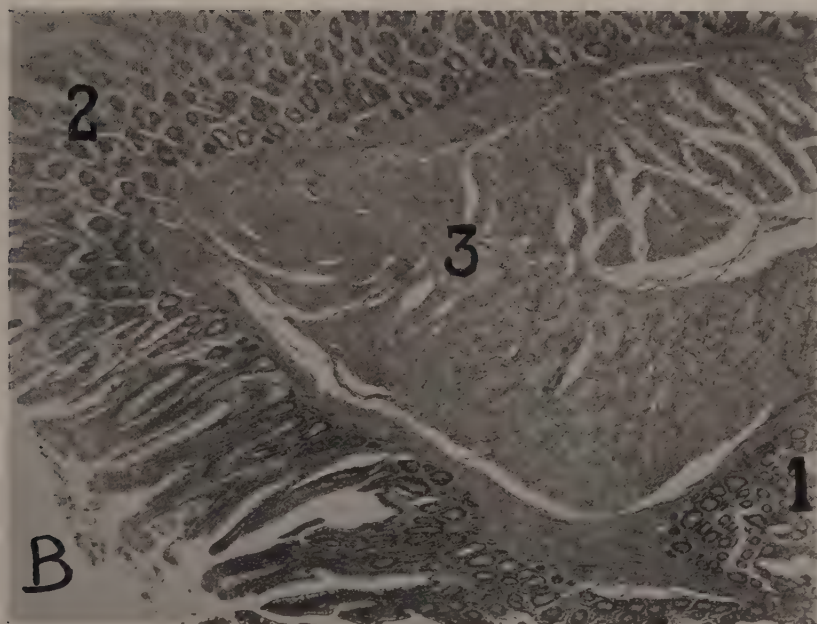
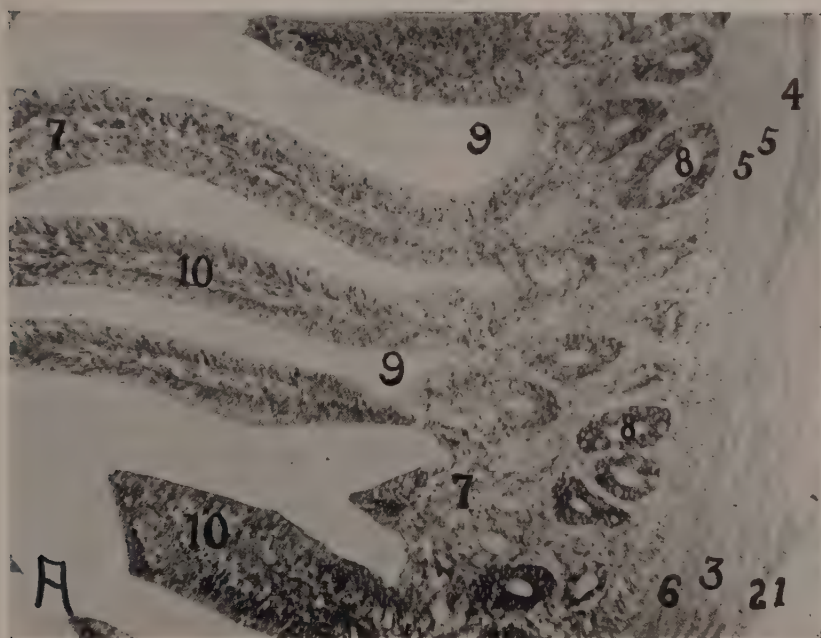


PLATE XXVII

Fig. A. Caecum, mid-portion, constricted. Hematoxylin-eosin. 200 x. 36 hours.

1. Epithelium
2. Villi
3. Tunica propria
4. Muscularis mucosae
5. Submucosa
6. Circular layer of lamina muscularis
7. Longitudinal layer of lamina muscularis
8. Serosa

Fig. B. Caecum, mid-portion, dilated. Hematoxylin-eosin. 200 x. 36 hours.

1. Epithelium
2. Villi
3. Tunica propria
4. Muscularis mucosae
5. Submucosa
6. Circular layer of lamina muscularis
7. Longitudinal layer of lamina muscularis

Plate XXVII

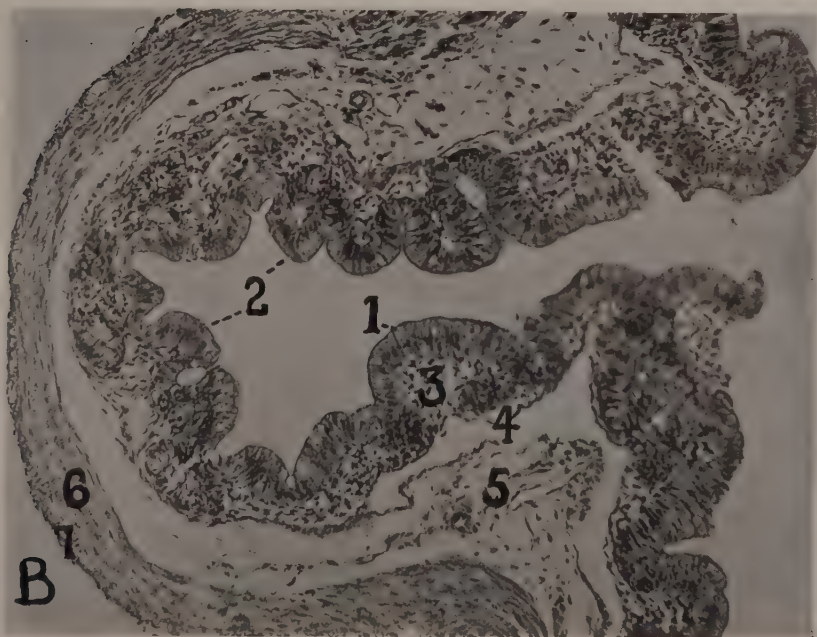
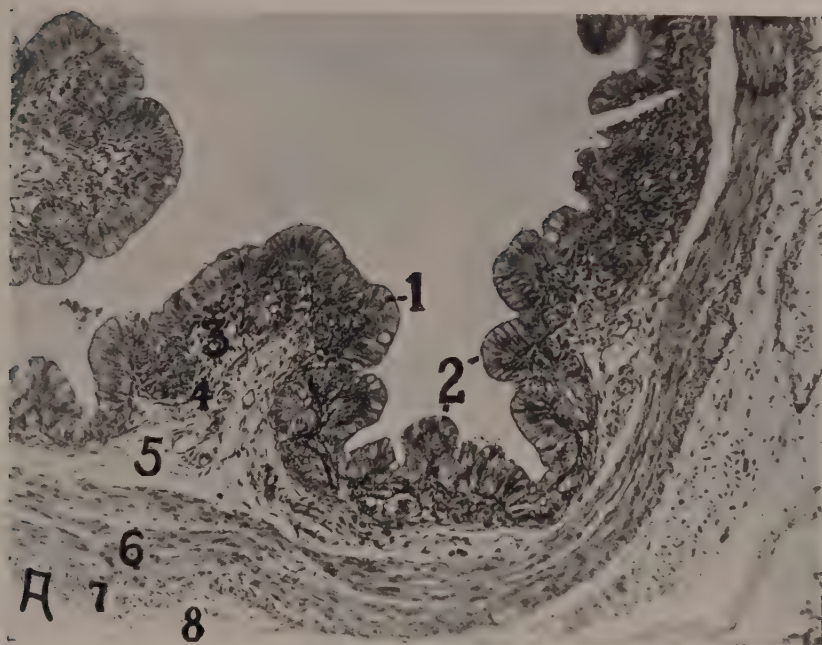


PLATE XXVIII

Fig. A. Lymphoid tissue in caecum (proximal portion). Longitudinal section Hematoxylin-eosin. 50 x. One and one-half years.

1. Longitudinal layer of lamina muscularis
2. Circular layer of lamina muscularis
3. Muscularis mucosae
4. Lymphoid tissue
5. Lymph nodules

Fig. B. Reticulum in glandular area of caecum. (Reticulum fine black lines.) Reticular stain. 200 x. One and one-half years.

1. Glands of Lieberkühn
2. Reticulum in tunica propria

Plate XXVIII

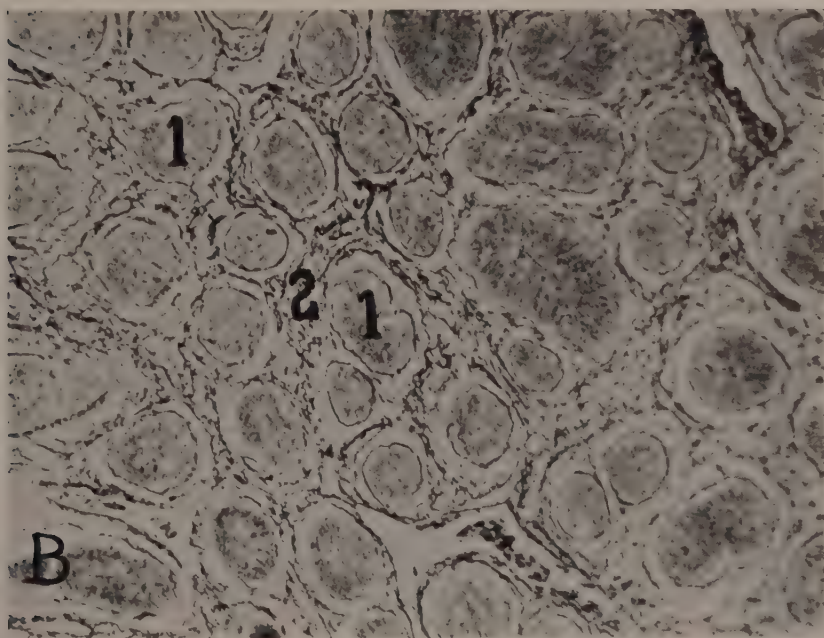
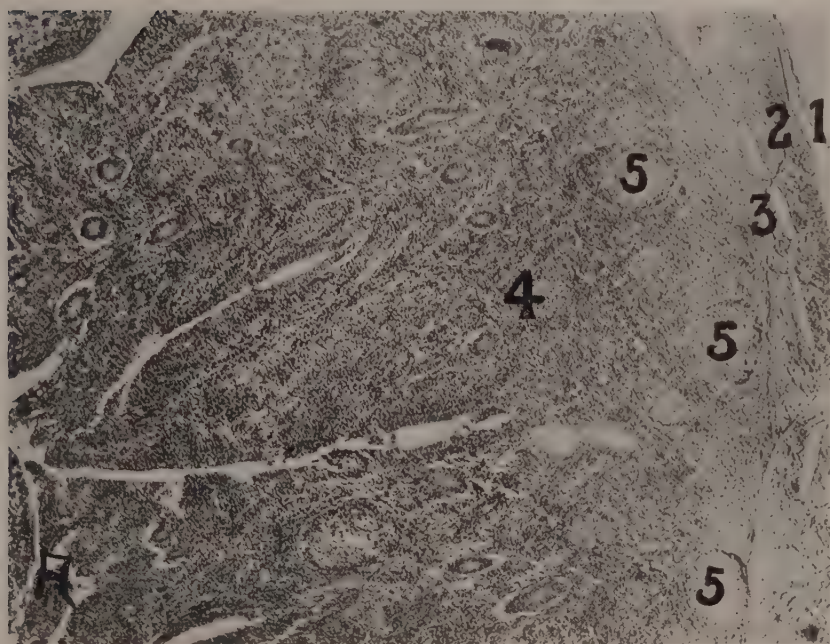


PLATE XXIX

Fig. A. Rectum. Hematoxylin-eosin. 200 x. 36 hours.

1. Goblet cells in the simple columnar epithelium
2. Villi
3. Tunica propria
4. Glands of Lieberkühn
5. Crypts of Lieberkühn
6. Circular layer of lamina muscularis
7. Longitudinal layer of lamina muscularis

Fig. B. Small and large intestine and both caeca. Hematoxylin-eosin. 25 x. One and one-half years.

1. Rectum
2. Small intestine
3. Caeca
4. Blood and lymph vessels and nerves

Plate XXIX

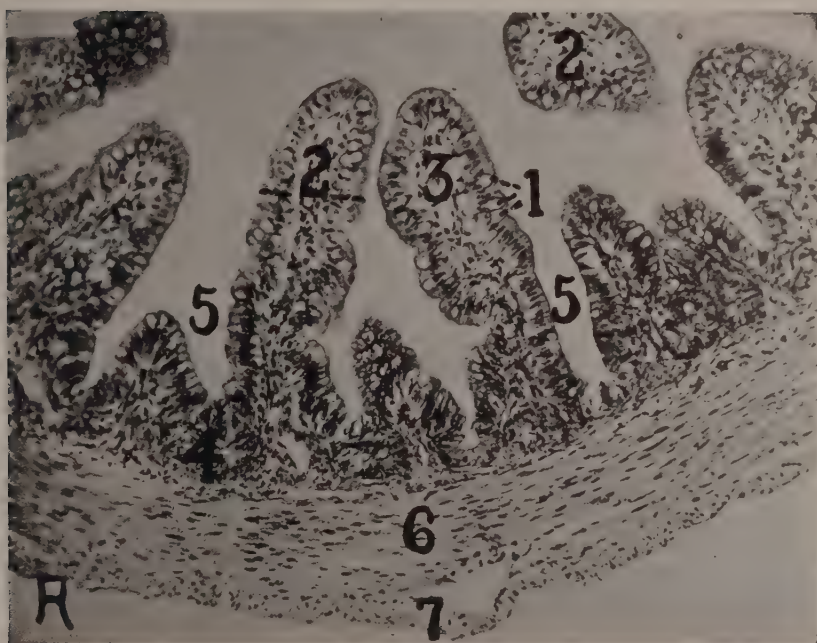


PLATE XXX

Fig. A. Cloaca showing a fold covering the entrance to the bursa cloacae. Hematoxylin-eosin. 25 x. 4 days.

1. Fold overhanging entrance to bursa cloacae
2. Dorsal wall of proctodaeum
3. Fold of wall of bursa cloacae
4. Dorsal wall of urodaeum
5. Ventral wall of urodaeum

Fig. B. Longitudinal section of the two anterior chambers of the cloaca. Hematoxylin-eosin. 25 x. 1 day.

1. Rectum
2. Coprodaeum
3. Urodaeum
4. Proctodaeum
5. Sphincter separating rectum from coprodaeum

Plate XXX

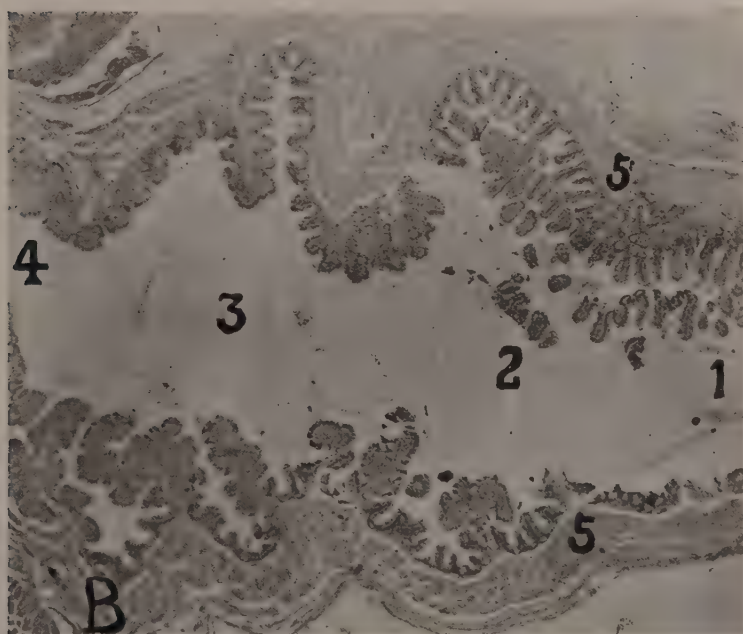
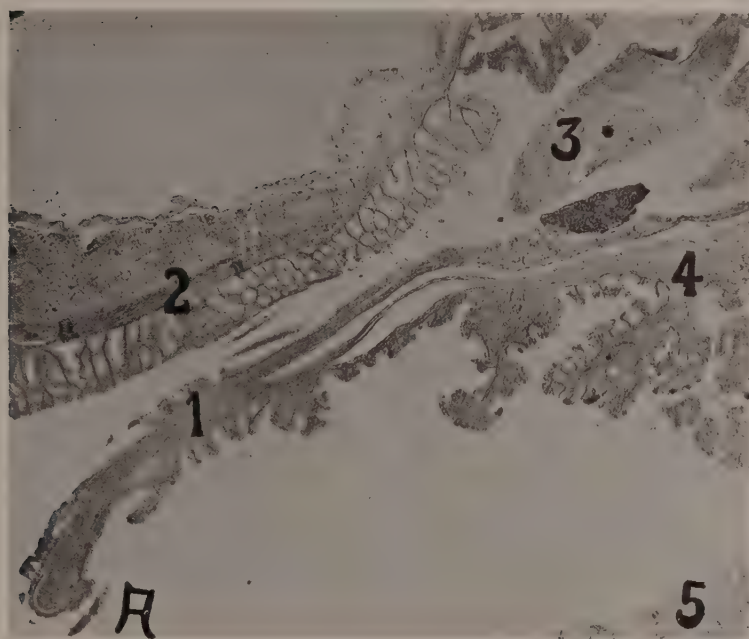


PLATE XXXI

Fig. A. Anal opening, longitudinal section. Hematoxylin-eosin. 50 x. 1 day.

1. Villi of proctodaeum
2. Dorsal lip of the anus
3. Ventral lip of the anus
4. Point at which the inner layer of muscle in the upper lip begins to change direction
5. Muscle of upper lip ending in a longitudinal arrangement
6. Muscle of lower lip arranged in an inner longitudinal and outer circular direction
7. All the muscle in the lower lip arranged in a longitudinal direction
8. Muscle of lower lip ending in a circular arrangement
9. Anal opening

Fig. B. Region in anal opening in which the stratified squamous epithelium of the anus changes to the simple columnar epithelium of the proctodaeum. Hematoxylin-eosin. 200 x. 1 day.

1. Epithelium of anal opening
2. Tunica prouria and submucosa
3. Muscle
4. Point at which stratified squamous epithelium changes to simple columnar

Plate XXXI

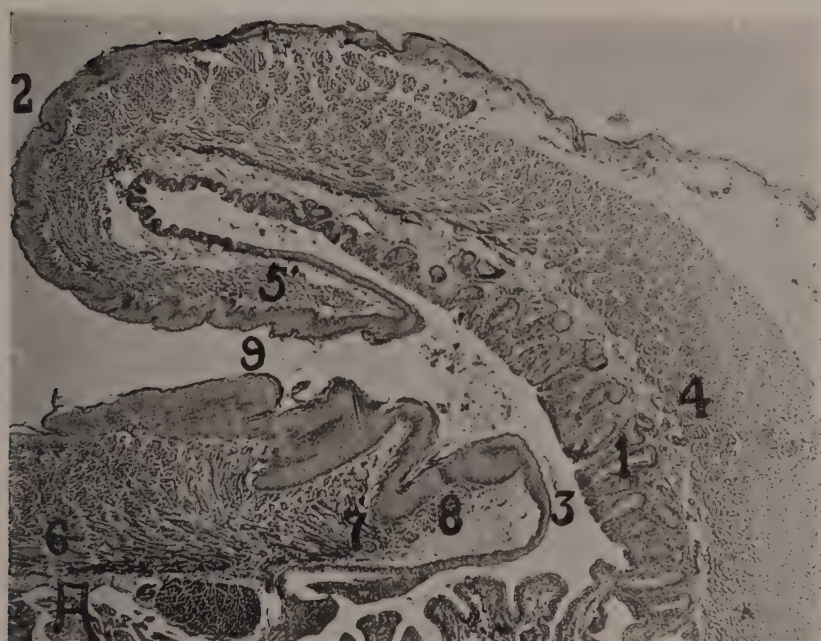


PLATE XXXII

Fig. A. Liver. Hematoxylin-eosin. 200 x. 2 years.

1. Central vein with sinusoids opening into it
2. Endothelial lining of central vein
3. Cords of liver cells

Fig. B. Liver showing portal canal. Note fat spaces in the liver parenchyma. Hematoxylin-eosin. 200 x. 8 days.

1. Portal vein
2. Bile ducts
3. Hepatic arteries
4. Lymph vessel
5. Liver parenchyma

Plate XXXII

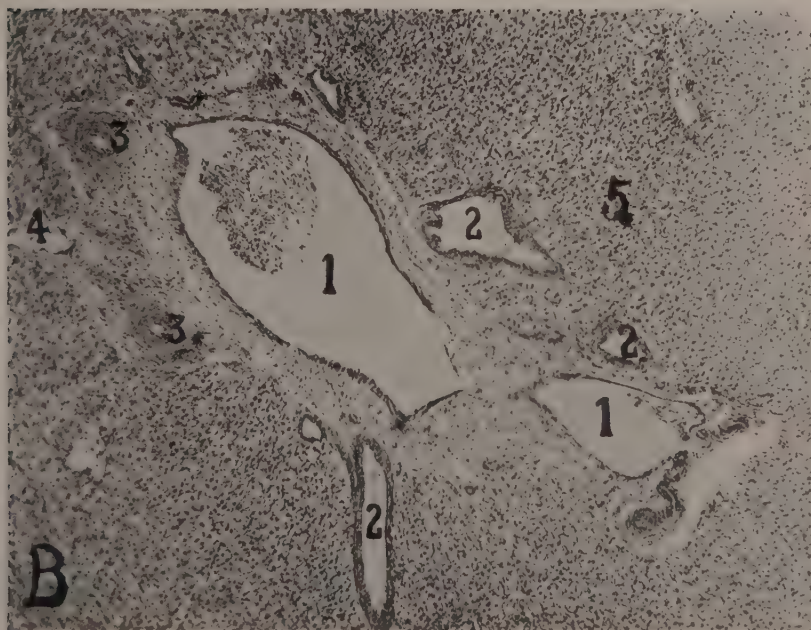
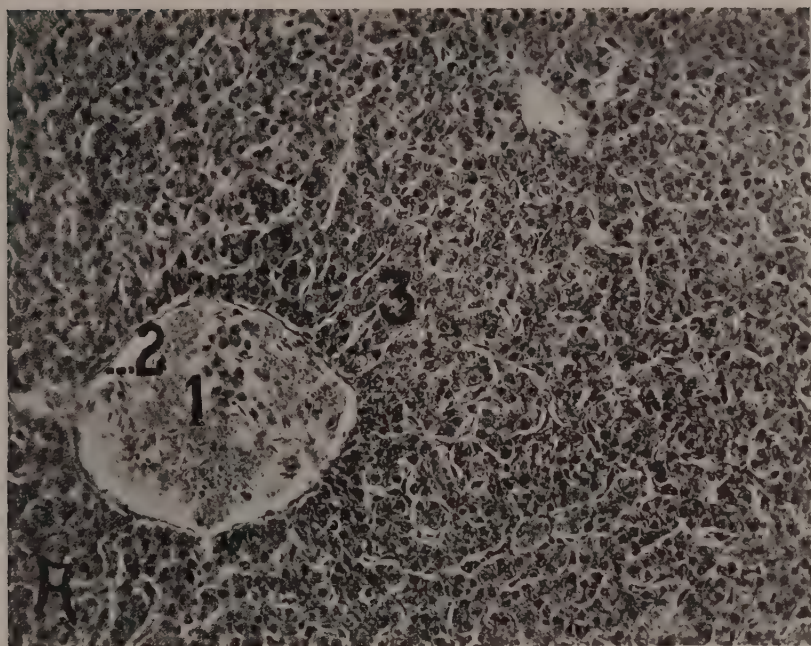


PLATE XXXIII

Fig. A. Liver stained for reticulum. Reticular stain. 800 x. One and one-half years.

1. Sinusoids
2. Reticulum (black lines)
3. Cords of liver cells

Fig. B. Liver stained for fat. Droplets appear dark. Scharlach R. 200 x. 10 days.

Plate XXXIII

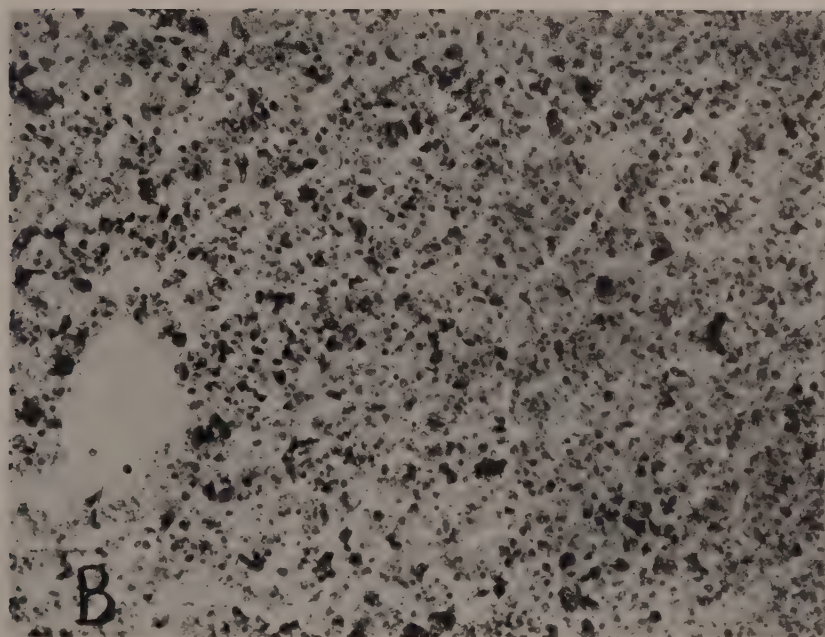
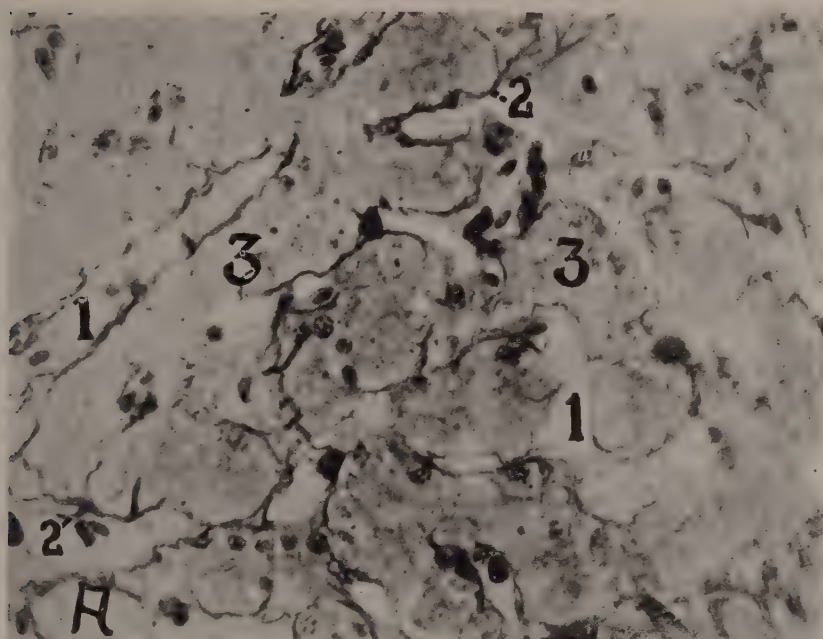


PLATE XXXIV

Figs. A—F. Liver stained for fat at 9, 12, 15, 18, 21, and 25 days. Scharlach B. 200 x.
Note gradual lessening of fat until on the 21st and 25th days it is confined to an area
around the vessels.

Fig. A— 9 days

Fig. D—18 days

Fig. B—12 days

Fig. E—21 days

Fig. C—15 days

Fig. F—25 days

Plate XXXIV

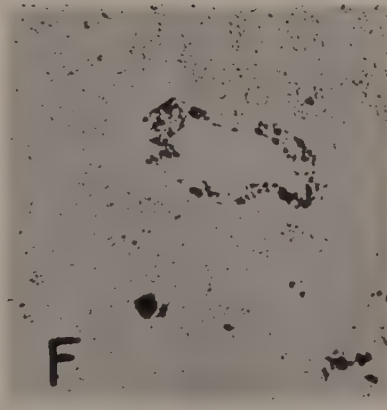
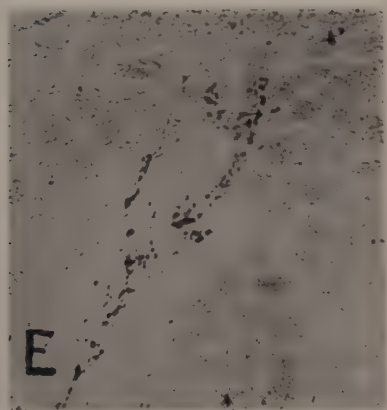
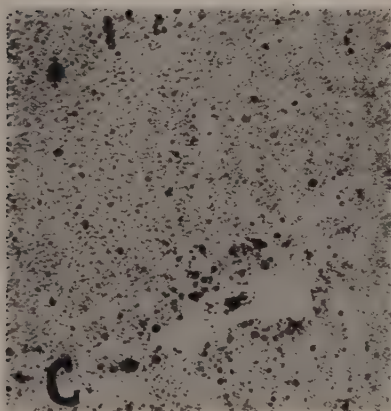
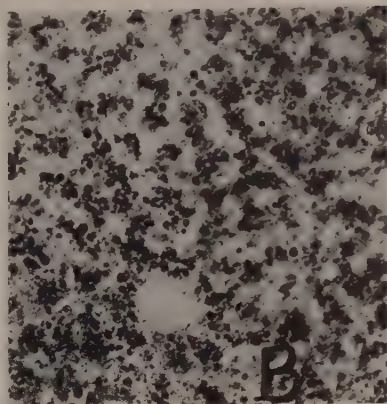
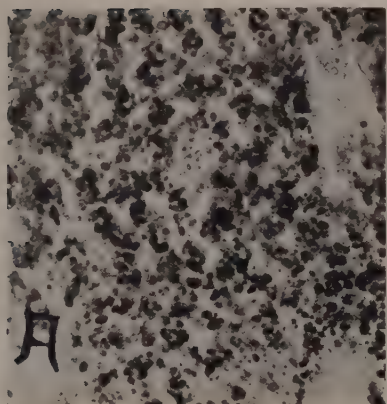


PLATE XXXV

Fig. A. Bile duct. Hematoxylin-eosin. 200 x. 2 days.

1. Lamina muscularis
2. Submucosa
3. Muscularis mucosae
4. Tunica propria
5. Villi lined with cylindrical epithelium

Fig. B. Pancreatic duct. Hematoxylin-eosin. 200 x. 5 months.

1. Longitudinal layer of the lamina muscularis
2. Circular layer of the lamina muscularis
3. Submucosa
4. Muscularis mucosae
5. Tunica propria
6. Villi lined with cylindrical epithelium

Plate XXXV

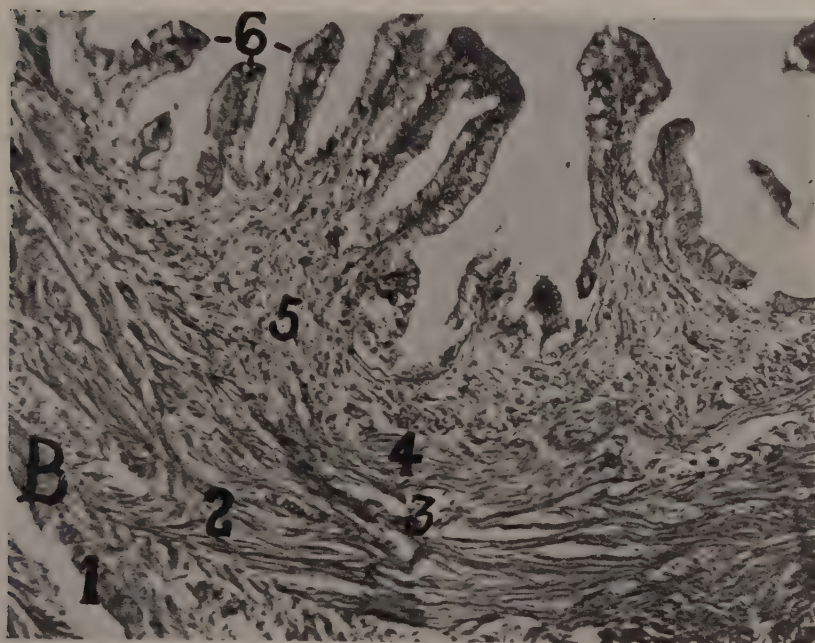
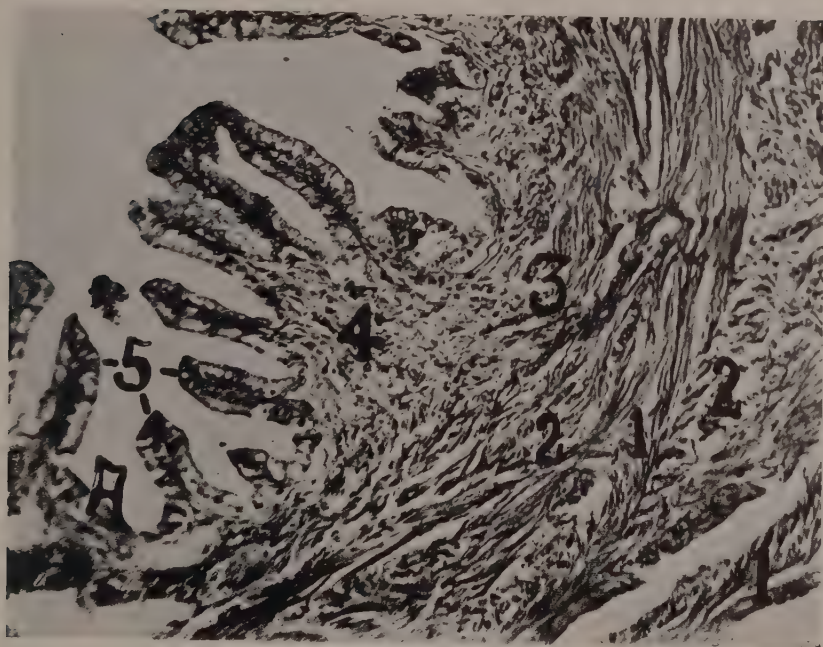


PLATE XXXVI

Fig. A. Pancreas. Hematoxylin-eosin. 200 x. 5 months.

1. Pancreatic acini
2. Islet of Langerhans
3. Collecting duct
4. Group of vessels and nerves
5. Peritoneal covering

Fig. B. Reticulum in pancreas. Reticular stain. 200 x. One and one-half years.

1. Acini
2. Reticulum (black lines)

Plate XXXVI

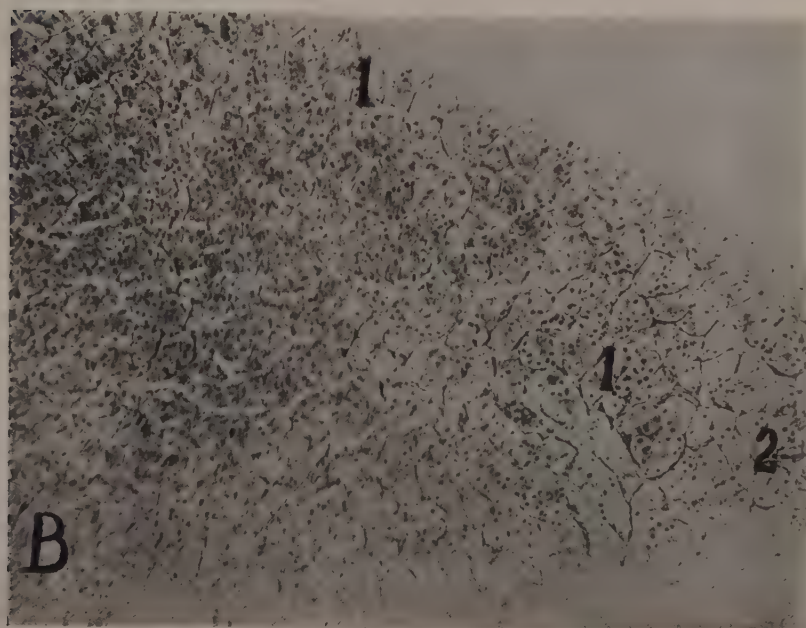
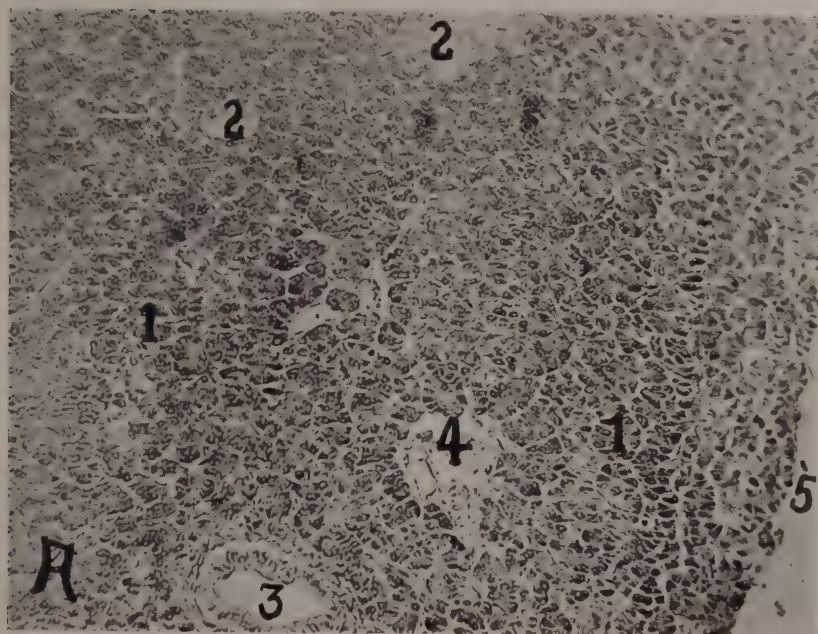


PLATE XXXVII

Fig. A. Fold in wall of the bursa cloacae. Hematoxylin eosin. 50 x. 7 months.

1. Trabeculae
2. Lymph follicles
3. Epithelium

Fig. B. Fold of the bursa cloacae. Hematoxylin-eosin. 50 x. Five and one-half months.

1. Epithelium
2. Epithelium joining the medullary portion
3. Medullary portion of a nodule
4. Cortical portion of a nodule

Plate XXXVII

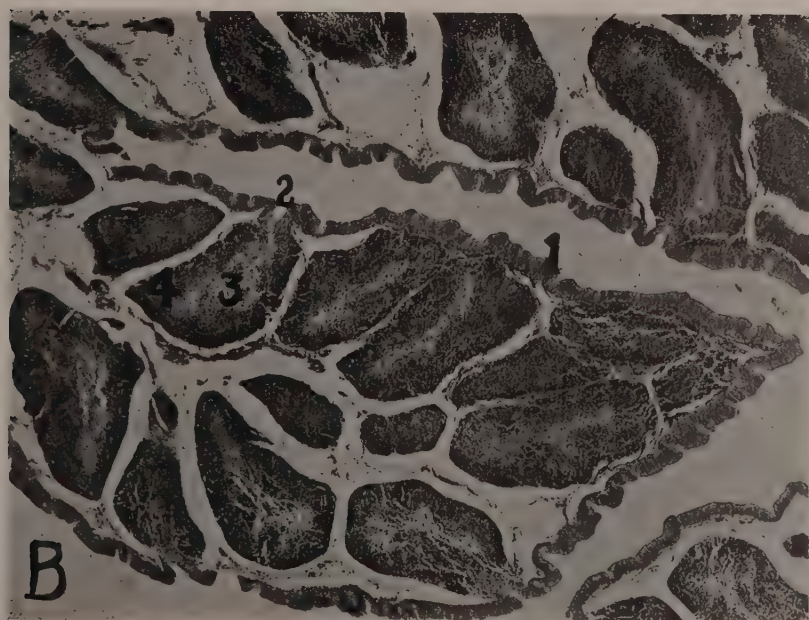
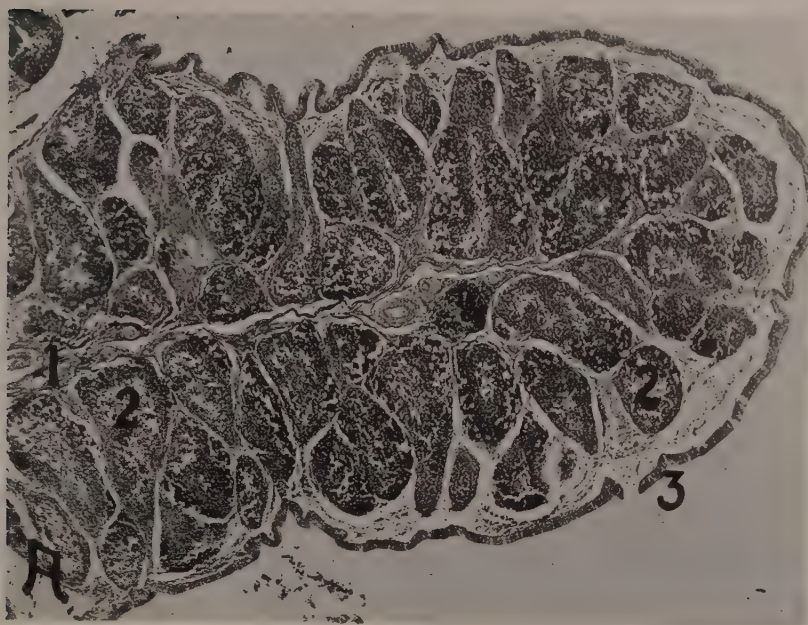


PLATE XXXVIII

Fig. A. Fold of the bursa cloacae showing reticulum. Reticular stain. 200 x. 7 months.

1. Medullary portion of lymph follicle
2. Cortical portion of lymph follicle
3. Reticular tissue at junction of medullary and cortical portions
4. Interfollicular septa

Fig. B. Reticulum separating cortical from medullary portions in a follicle of the bursa cloacae. Reticular stain. 800 x. 7 months.

1. Cortical portion
2. Medullary portion

Plate XXXVIII

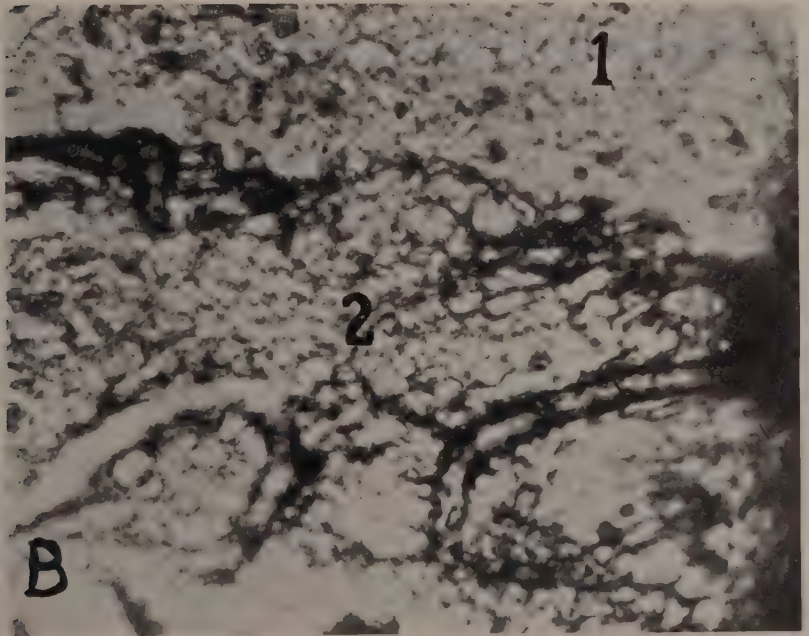
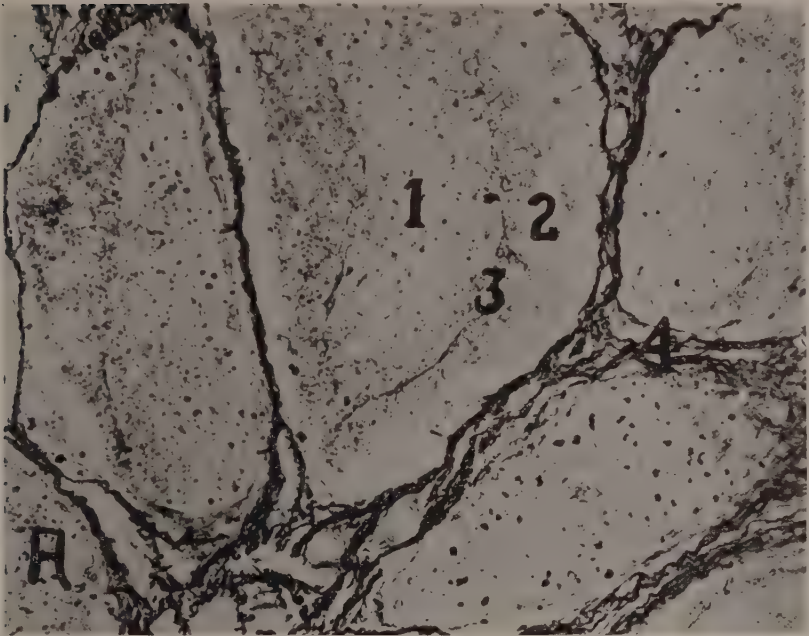


PLATE XXXIX

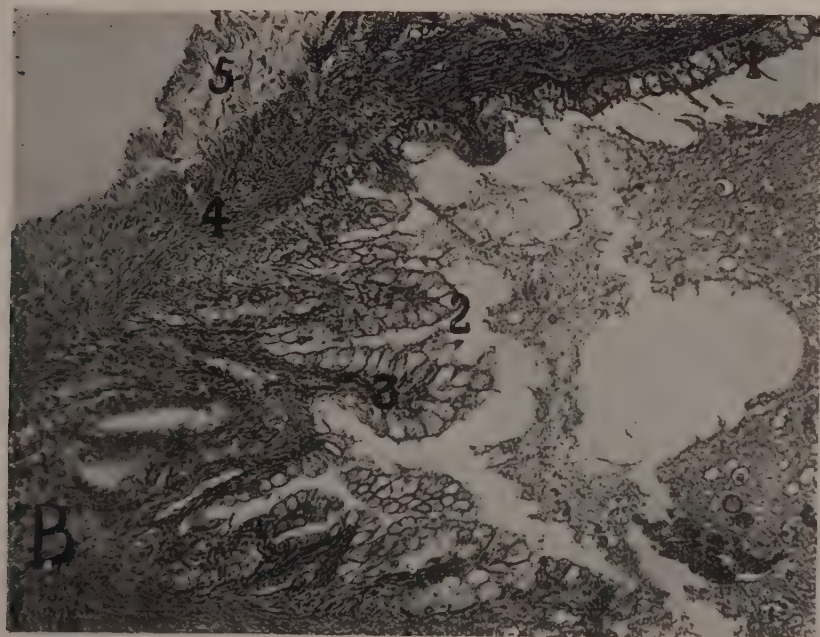
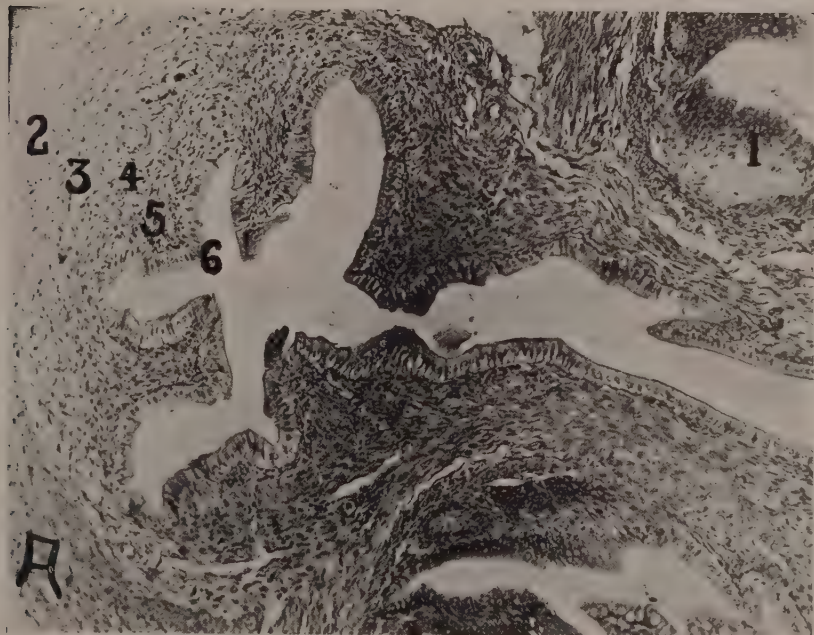
Fig. A. Yolk stalk. Hematoxylin-eosin. 200 x. 36 hours.

1. Intestinal mucosa
2. Lamina muscularis
3. Submucosa
4. Muscularis mucosae
5. Tunica propria
6. Columnar epithelium

Fig. B. Yolk sac. Hematoxylin-eosin. 200 x. 1 day.

1. Cuboidal epithelium of yolk stalk
2. Columnar epithelium of yolk sac
3. Folds in the mucous membrane
4. Fibrous connective tissue layer
5. Serosa

Plate XXXIX



THE STABILITY OF ETHYLENE GLYCOL IN ACID SOLUTIONS

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Attempts to prepare diols by hydrogenation at temperatures between 100° and 150° in acid solutions resulted in the disappearance of the substances subjected to the treatment, but no diols were produced. It was suspected that the diols might not be stable under these conditions. Since ethylene glycol is abundant and cheap, it was chosen for the first series of tests. At 107°, this diol was subjected to sulfuric acid in concentrations between 20 per cent and 35 per cent and to hydrochloric acid in concentrations between 3 per cent and 25 per cent. Using solutions containing 25 per cent sulfuric acid or 10 per cent hydrochloric acid the temperature was varied from 107° to 135°. Some experiments were conducted under hydrogen at a pressure of 1750 pounds per square inch.

EXPERIMENTAL

A determination was made by sealing a mixture composed of 25 cc. of ethylene glycol and 75 cc. of a solution of acid in a suitable container, and subjecting it to a chosen temperature. For the temperatures which produced low pressures, ordinary 150 cc. pressure bottles were used. For higher temperatures pyrex bomb tubes were used. When the effect of high pressures of hydrogen was investigated a glass container with a small opening was enclosed in a small autoclave before being heated. In one series of four experiments the mixtures which contained sulfuric acid were refluxed at their boiling points at atmospheric pressure. The pyrex bomb tubes were heated in a combustion furnace. All other containers were heated in an oil bath. The temperatures were maintained for three hours exclusive of the time required for heating and cooling, but the duration of the heating and cooling periods was made as short as possible, without danger of breaking the glass containers. The pressure of hydrogen used was that furnished by a commercial tank, 1750 pounds per square inch.

DETERMINATION OF GLYCOL

Nearly all of the decomposition products of ethylene glycol are insoluble in the glycol-acid-water mixture and the two layers were separated by means of a separatory funnel. The oily decomposition products were extracted with water several times and the extracts added to the glycol solution.

When the solution contained hydrochloric acid, lead carbonate was added until there was no further effervescence. The precipitated lead chloride was filtered from the cooled solution and washed with alcohol to remove any adhering glycol. Alcohol was added to the filtrate and the small additional precipitate was collected and washed with alcohol.

When the solution contained sulfuric acid, neutralization was effected

by means of sodium hydroxide. Much $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ crystallized out on standing. These crystals were filtered out and washed with alcohol. To the filtrate was added two to three volumes of alcohol. The additional precipitate was removed by filtration at low temperatures.

In either case the alcohol and water could be distilled from the glycol readily, but the glycol was too viscous to be removed easily from the distillation flask for measurement. The method chosen for the estimation of glycol was as follows: The volume of each alcohol-water-glycol mixture was carefully measured and the mixture was put into a distillation flask of suitable size. (The volume of each solution was approximately 250 cc.) The distillate was collected in a graduated container until the temperature

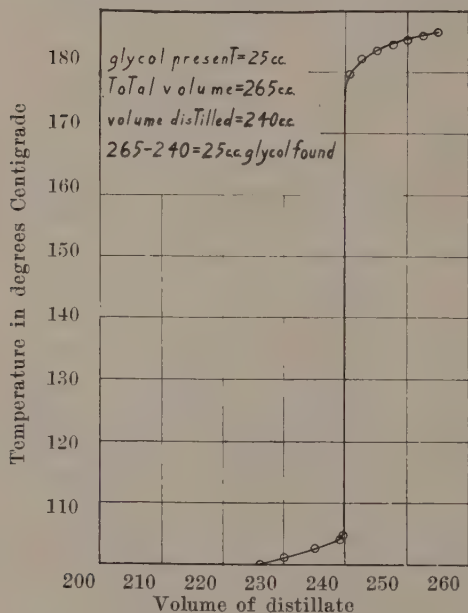


Fig. 1. The distillation of a mixture of water alcohol and ethylene glycol.

of the vapor reached 100° . At that point an accurate reading of the volume of the distillate was made. Distillation was continued. A record of the total volume of distillate and the corresponding temperature of the vapor was made until the temperature of vapor reached 188° , the boiling point of ethylene glycol. The total volume of distillate was plotted against the temperature of the vapor in the distilling flask. The volume of water and alcohol was estimated by reading the volume indicated by the mid point of the inflection in the curve between 100° and 188° . The volume of the undecomposed glycol was found by difference.

Figure 1 represents graphically the data taken while distilling a solution known to contain 25 cc. of ethylene glycol in enough water and alcohol to produce a total volume of 265 cc.

Data from other experiments with varying known proportions of alcohol, water and glycol produced curves so nearly like that in figure 1 that they could not be distinguished from it if drawn on the same sheet.

The distillation of the last traces of water from the glycol produced violent bumping which was not stopped by: broken unglazed plate, boiling stones, glass bead, glass tubes, or any such devices. Bumping was eliminated, and rapid and quiet boiling was made possible by distilling from a three-necked flask equipped as follows: A distilling column was fitted to one side neck. The second side neck was stoppered while distillation was in progress and used for the introduction of portions of the sample to be distilled. The shaft of a motor stirrer sealed by a mercury seal was fitted to the middle neck. To the shaft was fastened a glass rod whose lower end had been flattened and recurved in such way that it rotated close to the bottom of the flask and produced a rising current of liquid.

EXPERIMENTAL RESULTS

The curves in figure 2 represent the decomposition of ethylene glycol in the presence of varying concentrations of hydrochloric acid or of sulfuric acid at 107° or in the presence of four concentrations of sulfuric acid refluxed at their boiling points under atmospheric pressure. The duration of each experiment was three hours.

In figure 3 the percentage of ethylene glycol decomposed in three hours is plotted against the temperature at which the experiment was carried out. One curve represents the decomposition in a solution which contained 10 per cent hydrochloric acid; and the second that in a solution which contained 25 per cent sulfuric acid.

In figure 4 the data is the same as that represented in figure 3 but instead of the percentage of acid, the abscissas represent the activities for the corresponding concentrations of the acids as indicated by the freezing point data, taken from the International Critical Tables.

Some experiments were made using, at different times, hydrochloric acid and sulfuric acid and imposing 1750 pounds pressure of hydrogen per square inch. In all such cases the percentage of the glycol decomposed was the same as when no pressure of hydrogen was imposed.

DISCUSSION

Ethylene glycol is quite unstable in the presence of even small concentrations of hydrochloric acid. As the concentration of acid increases the rate of decomposition of the glycol increases rapidly. When the concentrations are equal, hydrochloric acid is more effective than sulfuric acid in effecting the decomposition of ethylene glycol. This is true regardless of whether percentage composition, normality, or activity as indicated by lowering of freezing point is used as the basis for measuring the concentrations.

Rise in temperature is accompanied by a rapid increase in rate of decomposition of the glycol in the presence of either acid. Over the greater part of the range studied the increase of rate of decomposition with rise of temperature is greater for solutions containing sulfuric acid than for those containing hydrochloric acid. Hydrogen is not evolved during the decomposition of ethylene glycol and its presence would not be expected to affect the rate of decomposition. The experiments verify this expectation.

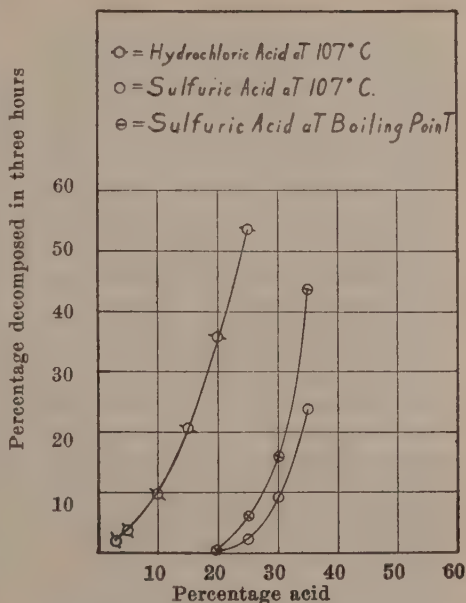


Fig. 2. The decomposition of ethylene glycol in the presence of acids at constant temperature.

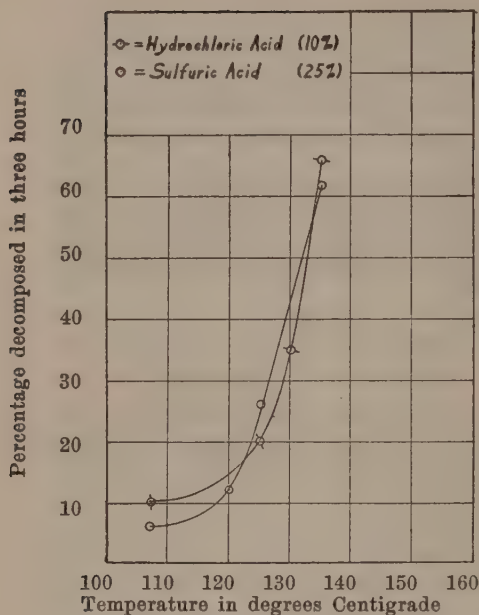


Fig. 3. The decomposition of ethylene glycol in the presence of acid at various temperatures.

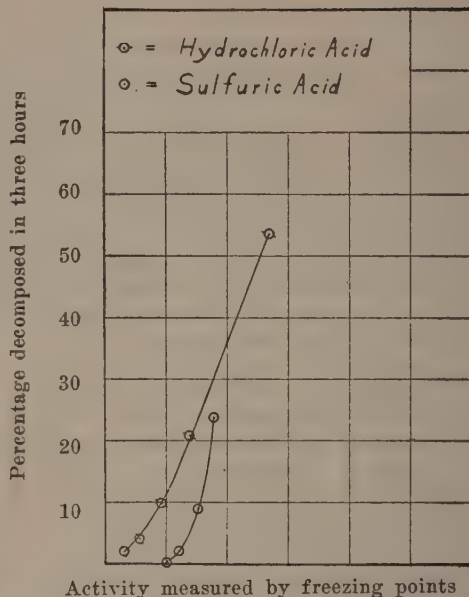


Fig. 4. The decomposition of ethylene glycol as related to activities of acids.

SUMMARY

1. At 107°, sulfuric acid at concentrations below 20 per cent by weight, produces little or no decomposition of ethylene glycol, but, at concentrations greater than 20 per cent it produces decomposition. The rate of decomposition increases rapidly with increasing concentration of acid, 40 per cent acid decomposing about 50 per cent of the glycol in three hours at 107°.

2. At the same temperature, decomposition with hydrochloric acid begins at very low concentrations and reaches a value of 54 per cent at a concentration of 10 per cent acid.

3. The effect of temperature on decomposition, in the case of both acids, is very great: a change of from 107° to 135°C., bringing about an increase of 60 per cent in decomposition, in the case of sulfuric acid, and 56 per cent with hydrochloric acid.

APPROXIMATE SOLUTIONS OF TWO-DIMENSIONAL ELASTIC PROBLEMS

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Most problems in the dynamics of continuous media ultimately reduce to the solving of a boundary value problem in which the equilibrium situation is represented by a differential equation. This boundary value problem is said to be solved if a solution of the given differential equation can be found such that it attains the prescribed value on the boundary of the region in question. In most cases the differential equation is a linear partial differential equation of second or higher order, and such a solution is desired that the function and certain relations shall be satisfied at the boundary. Frequently, the difficulties encountered in meeting these requirements are so great that other exact or approximate methods are resorted to.

When the differential equation possesses the property of being self-adjoint, as is generally the case in problems of physical interest, it may be associated with a variational problem. An equivalent exact formulation of the boundary value problem is the construction of an integral, usually shown to be some form of an energy integral, which is to be minimized and also to satisfy the required conditions on the boundary. This becomes a problem in the Calculus of Variations, in which one has to find from among all the admissible functions with the necessary differentiability conditions, a function which minimizes the given integral and attains the necessary boundary values. The classical method employed in solving this problem is to find the first variational derivative. As a consequence of the vanishing of this variational derivative, there results a first necessary condition which the required function must satisfy, namely the Euler-Lagrange equation, which is precisely the equilibrium equation of the boundary value problem.

In contrast to the classical method, direct methods of solving these problems are of great interest. The important method of approximation invented by Lord Rayleigh[1]* and developed by W. Ritz[2], has been used by many writers[3] to obtain approximate solutions of many important problems. In Ritz's method a boundary value problem for a differential equation $L(u)=0$, is replaced by a variation problem in which a certain integral $I[u]$ is to be made a minimum, the unknown function $u(x,y)$ being subject to certain supplementary conditions which are usually linear boundary conditions. The function $u_m(x,y)$, used by Ritz as an approximation for $u(x,y)$, is generally not a solution of the differential equation, but it does satisfy the boundary conditions for all values of the arbitrary parameters which it contains. The result is that when an integral I_m is obtained from using the approximating function u_m , it is greater than the true

* References are indicated by number in [].

minimum value I_d of I , even when the arbitrary parameters are chosen so as to make I_m as small as possible.

When the approximation function u_m can be chosen as a linear combinations of a normally orthogonalized set of functions, the convergence of I_m to I_d , as well as u_m to u can be shown. It was shown by Ritz, moreover, that sometimes the functions occurring in the exact solution of one problem may advantageously be used in the approximate solution of another problem. A variation of Ritz's method has been recommended by Hencky[4] and Goldsbrough[5]; it has the advantage of indicating a reason why in the limit the function u_m should satisfy $L(u)=0$.

Another direct method for solving a variational problem is due to E. Trefftz[6]. The contrast of this method with that of Ritz lies in the fact that when an integral I_m of I is calculated from an approximate function u_m such that $L(u_m)=0$, but with less restricted boundary conditions, then I_m approaches I_d from below. This method was applied first by Trefftz to Dirichlet's integral $I[u]$ for a potential function $\nabla^2 u=0$, and direct application made to the torsion problem [6]. Extensions were made by R. Courant[7] and K. Friedrichs[8]. The former enunciated a principle applicable to variation problems, which states that the actual minimum of an integral cannot increase when the boundary conditions become less restrictive or if none are imposed whatever. For example, if it is required that $u(s) - f(s)=0$ on a boundary, the boundary condition may be loosened by merely requiring that the integral of $q(s)[u(s) - f(s)]=0$. Hence, if q satisfy $L(q)=0$, it may make it possible to find simple solutions of the differential equation satisfying the loosened boundary conditions. Friedrichs has pointed out that a new integral J may be constructed, free from natural boundary conditions and such that the maximum of J is equivalent to the minimum value of I . By Courant's principle any approximation J_m for J is smaller than I_d . N. M. Bashu[9] and the writer [10] have applied the Trefftz method to special cases of the torsion problem.

By an application of both the Ritz and Trefftz methods, an upper and a lower bound of the true minimum is established. In either case the one method provides an error criterion for the approximation obtained by the other method. In general it may be said that the Ritz method affords a more rapidly convergent method than does the Trefftz method even though the choice of the approximating functions is more limited. R. Courant[11] has shown that the rapidity of convergence of a method of approximation can often be improved by modifying the variational problem, introducing higher derivatives in such a way that the Eulerian equation of the problem is satisfied whenever the original differential equation is satisfied. This device is useful in applications of Trefftz's method.

STRESS PROBLEM

Consider the boundary value problem of finding a biharmonic function $w(x,y)$ satisfying

$$(1) \left\{ \begin{array}{l} L(w)=\nabla^4 w = \frac{\partial^4 w}{\partial x^4} + 2 \frac{\partial^4 w}{\partial x^2 \partial y^2} + \frac{\partial^4 w}{\partial y^4} = 0 \text{ in } R, \\ w=f(s) \quad ; \quad \frac{\partial w}{\partial n} = g(s) \quad \text{on } \Gamma, \end{array} \right.$$

in a simply connected region R , with a regular [12] contour Γ , on which $f(s)$ and $g(s)$ are analytic functions of the arc length s . It is assumed that w possesses derivatives of at least the fourth order. The normal derivative is taken along the outward normal and s increases in such a way that R is circumscribed in counter clockwise fashion.

The equivalent variation problem is the problem of finding among all the admissible functions $w = w(x, y)$ which possess the necessary differentiability conditions, a function which satisfies (1) and minimizes the integral

$$(2) \quad I[w] = \frac{1}{2} \iint (\nabla^2 w)^2 dx dy = \frac{1}{2} \iint \left[\frac{\partial^2 w}{\partial x^2} + \frac{\partial^2 w}{\partial y^2} \right]^2 dx dy.$$

That this integral has a meaning is seen from the fact that it has a non-negative lower bound. Postulating the existence of the function w , one may show analytically that a non-negative lower bound of (2) exists. Such a proof is given by K. Friedrichs [3]. If the invariance of the operator is taken into consideration, in any given problem, one usually finds an interpretation for the integrand of (2) and is thus able to accept the existence of a minimum.

Ritz's Method. As an m th approximation for w , one may choose a function of the form

$$(3) \quad v_m(x, y) = f(x, y) + \sum_{j=1}^m c_j q_j(x, y),$$

such that all q_j vanish on Γ , with $\frac{\partial f}{\partial n} = 0$, and $\sum_{j=1}^m c_j \frac{\partial q_j}{\partial n} = g(s)$ on the contour Γ of R . Then the parameters c_j of (3) are determined so that

$$I[v_m] = \iint (\nabla^2 v_m)^2 dx dy$$

shall be a minimum. From the condition for a minimum of $I[c_1, c_2, \dots, c_m]$, there result m linear non-homogeneous equations,

$$(4) \quad \frac{\partial I}{\partial c_i} = 0, \quad (i = 1, 2, \dots, m.)$$

for the determination of the m parameters.

That the approximation function of (3) yields a larger minimum of (2) than the true minimum I_a , may be shown as follows. Let the error be denoted by

$$(5) \quad \sigma_m(x, y) = v_m(x, y) - w(x, y).$$

Then by the conditions imposed on (3), $\sigma_m(x, y)$ vanishes on Γ , since $w = f(s)$. From (5) it follows that

$$\begin{aligned} (6) \quad \iint (\nabla^2 v_m)^2 dx dy &= \iint (\nabla^2 \sigma_m + \nabla^2 w)^2 dx dy \\ &= \iint [(\nabla^2 \sigma_m)^2 + 2(\nabla^2 \sigma_m)(\nabla^2 w) + (\nabla^2 w)^2] dx dy \\ &= \iint (\nabla^2 \sigma_m)^2 dx dy + \iint (\nabla^2 w)^2 dx dy. \end{aligned}$$

Since the first integral in the right member of (6) is positive, $I[v_m] > I[w]$.

It remains to show that

$$\iint (\nabla^2 \sigma_m) (\nabla^2 w) \, dx dy = 0.$$

By Green's formula,

$$\begin{aligned} (7) \quad & \iint (\nabla^2 \sigma_m) (\nabla^2 w) \, dx dy \\ &= \int \left[(\nabla^2 w) \left(\frac{\partial \sigma_m}{\partial n} \right) - \sigma_m \frac{\partial (\nabla^2 w)}{\partial n} \right] ds + \iint \sigma_m \nabla^4 w \, dx dy \\ &= 0 \end{aligned}$$

since by (3), and (5), σ_m and $\frac{\partial \sigma_m}{\partial n}$ vanish on the boundary and $\nabla^4 w = 0$.

This concludes the proof.

When the functions q_i of (3) can be constructed from linear combinations of an orthogonalized set of functions, the error function of (5) will vanish when m increases indefinitely and

$$(8) \quad \lim_{m \rightarrow \infty} I[v_m] = I[w].$$

Trefftz Method. Suppose that one does not attempt to satisfy the boundary values of (1) at all points, but rather that the following conditions containing the $2m$ unknown functions $p_i(s)$ and $q_i(s)$ be fulfilled:

$$(9) \quad J_i = \int [w - f(s)] p_i(s) ds + \int \left[\frac{\partial w}{\partial n} - g(s) \right] q_i(s) ds = 0.$$

($i = 1, 2, \dots, m$.)

Consider the problem of minimizing (2) subject to the m conditions of (9). The process of Lagrangean multipliers is employed and one forms the function

$$(10) \quad J_0[w, \lambda_1, \dots, \lambda_m] = I[w] + \sum_{i=1}^m \lambda_i J_i,$$

which is to be minimized. As a necessary condition the first variation of J_0 is to vanish for all admissible variations of w . In this paper the weak variations, or arbitrary continuous variations of the surface and its normal derivative in the region R and on its contour, are considered as admissible.

$$\begin{aligned} (11) \quad \delta J_0 &= \iint (\nabla^2 w) \nabla^2 (\delta w) \, dx dy \\ &+ \sum_{i=1}^m \lambda_i \int \left[(\delta w) p_i(s) + q_i(s) \frac{\partial (\delta w)}{\partial n} \right] ds = 0. \end{aligned}$$

Applying Green's theorem to the double integral of (11), one obtains

$$\begin{aligned} & \int \left[\nabla^2 w \frac{\partial (\delta w)}{\partial n} - \delta w \frac{\partial (\nabla^2 w)}{\partial n} \right] ds + \iint (\delta w) \nabla^4 w \, dx dy \\ &+ \sum_{i=1}^m \lambda_i \int \left[(\delta w) p_i(s) + q_i(s) \frac{\partial (\delta w)}{\partial n} \right] ds = 0. \end{aligned}$$

For arbitrary variations of w as specified, we have

$$(12) \quad \nabla^4 w = 0, \text{ at all points in } R,$$

and

$$\left. \begin{aligned} (13) \quad \nabla^2 w + \sum_1^m \lambda_1 q_1 &= 0 \\ (14) \quad \frac{\partial(\nabla^2 w)}{\partial n} - \sum_1^m \lambda_1 p_1 &= 0 \end{aligned} \right\} \text{along the boundary } \Gamma.$$

There are still $2m$ conditions at our disposal. Hence if we choose $q_1(x, y)$ and $p_1(x, y)$ such that they satisfy

$$\left. \begin{aligned} (15) \quad \nabla^2 q_1(x, y) + q_1(x, y) &= 0, \\ (16) \quad p_1(x, y) - \frac{\partial(\nabla^2 q_1)}{\partial n} &= 0. \end{aligned} \right\} (i=1, 2, \dots, m.)$$

respectively, then we may construct $w(x, y)$ in the form

$$(17) \quad w_m(x, y) = \sum_1^m \lambda_1 q_1(x, y)$$

and all the conditions of (12), (13) and (14) are satisfied if

$$(18) \quad \nabla^4 q_1(x, y) = 0.$$

From (9) the m conditions, for determining the λ_1 , are

$$(19) \quad \int [w_m - f(s)] \frac{\partial(\nabla^2 q_1)}{\partial n} ds - \int \left[\frac{\partial w_m}{\partial n} - g(s) \right] \nabla^2 q_1 ds = 0. \\ (i=1, 2, \dots, m.)$$

Another method of obtaining the results of (19) will be given to prove the following theorem.

THEOREM: *The necessary and sufficient condition that (17) and (19) will solve the variation problem (2) is that (18) hold.*

That it is necessary has been shown. To establish the sufficiency, one may assume (18) and proceed to determine λ_1 , ($i=1, 2, \dots, m$) so that the integral of the square of the Laplacean of the error is as small as possible. If w_m is given by (17) and (18) and

$$\sigma_m(x, y) = w(x, y) - w_m(x, y)$$

then

$$E_m = \iint (\nabla^2 \sigma_m)^2 dx dy = \iint [\nabla^2 w - \nabla^2 w_m]^2 dx dy$$

is to be a minimum. Then

$$(20) \quad dE_m = \sum_{i=1}^m \frac{\partial E_m}{\partial \lambda_1} d\lambda_1 = 0$$

for all choices of λ_1 . Hence

$$(21) \quad \frac{\partial E_m}{\partial \lambda_1} = 2 \iint (\nabla^2 w - \nabla^2 w_m) \nabla^2 \left(\frac{\partial w_m}{\partial \lambda_1} \right) dx dy$$

$$\begin{aligned}
&= 2 \iint (\nabla^2 w - \nabla^2 w_m) \nabla^2 \varrho_i \, dx dy \\
&= 2 \int \left[\nabla^2 \varrho_i \frac{\partial (w - w_m)}{\partial n} - (w - w_m) \frac{\partial \nabla^2 \varrho_i}{\partial n} \right] ds \\
&= 0, \quad (i = 1, 2, \dots, m.)
\end{aligned}$$

Equations (21) which are the same as (19) provide m linear nonhomogeneous equations for determining the multipliers λ_i since $f(s)$ and $g(s)$ are defined on Γ .

It remains to show that $I[w_m] \geq I[w]$. This is a direct consequence of Courant's principle, since the boundary conditions of (19) are less restrictive than (1). It may also be established as a consequence of the classical method of expansion in a series of particular solutions as follows:

$$\begin{aligned}
(22) \quad I[w] &= \iint (\nabla^2 w)^2 \, dx dy \\
&= \iint [\nabla^2 (w_m + \sigma_m)]^2 \, dx dy \\
&= \iint [(\nabla^2 w_m)^2 + 2(\nabla^2 w_m)(\nabla^2 \sigma_m) + (\nabla^2 \sigma_m)^2] \, dx dy \\
&= \iint [(\nabla^2 w_m)^2 + (\nabla^2 \sigma_m)^2] \, dx dy \\
&\geq I[w_m],
\end{aligned}$$

since

$$\begin{aligned}
\iint (\nabla^2 w_m)(\nabla^2 \sigma_m) \, dx dy &= \int \left[\nabla^2 w_m \frac{\partial \sigma_m}{\partial n} - \sigma_m \frac{\partial (\nabla^2 w_m)}{\partial n} \right] ds \\
&= \int \left[\sum_1^m \nabla^2 \varrho_i \left(\frac{\partial w}{\partial n} - \frac{\partial w_m}{\partial n} \right) - (w - w_m) \frac{\partial (\nabla^2 \varrho_i)}{\partial n} \right] ds \\
&= 0.
\end{aligned}$$

The last line integral vanishes by (21), and this concludes the proof.

Thus a function, satisfying the biharmonic $\nabla^4 = 0$, subject to becoming $f(s)$ on the boundary and its normal derivative assuming $g(s)$ on the boundary, may be approximated by either of the forms (3) or (17) with the parameters determined by (4) and (19) respectively, and the true minimum of (2) will be bounded by $I[v_m] \geq I[w] \geq I[w_m]$.

APPLICATIONS

For practical utility each method may possess certain advantages over the other. In general it may be said that the Ritz method affords a more rapidly convergent method than does the Trefftz method even though the choice of approximating functions is more limited. In the latter, while the empirical convergence is slow, the greatest error of the approximation lies near the boundary. In the former while the boundary conditions are satisfied, the differential equation is not satisfied anywhere within the region R .

As an interpretation of these ideas when applied to a boundary value problem in elasticity, we may choose an approximation function satisfying the equation of equilibrium but not fulfilling the requirements on the boundary. In other words one may prefer the case that the differential equation represents more adequately the true state of affairs within the

elastic member itself, than do the functional boundary relations describe the actual values over the boundary of that member. This is particularly true when homogeneous elastic structural members are pinned and joined to one another.

Stress Problem. The solution of two dimensional stress problems in elasticity by means of Airy's Stress Function F , satisfying the biharmonic equation $\nabla^4 F = 0$ and the given boundary conditions, is equivalent to the determination of the form F which makes the strain energy intergral

$$(23) \quad V = \frac{1}{2E} \int \int [(\nabla^2 F)^2 - 2(1 - \mu)(F_{xx} F_{yy} - F_{xy}^2)] dx dy$$

taken over the volume of the plate or prism a minimum. When the boundary conditions are prescribed loads, and not prescribed displacements, we have the case of a plane stress distribution, and the partial derivatives F_{xx} , F_{yy} , and F_{xy} are known on the boundary. E and μ are material constants, Young's modulus and Poisson's ratio respectively. Since the latter part of the intergrand of (23) may be written

$$F_{xx} F_{yy} - F_{xy}^2 = (F_x F_{yy})_x - (F_x F_{xy})_y = - (F_y F_{xy})_x + (F_y F_{xx})_y,$$

the surface integral may be transformed to line intergrals and hence dependent upon the boundary values of the given stress problem. Thus the problem of minimizing (23) becomes the problem of equation (2) since the Euler-Lagrange condition remains $\nabla^4 F = 0$.

By Ritz's method one may write a suitable function in the form

$$F = F_0 + \sum_{i=1}^m \alpha_i F_i$$

in which F_0 satisfies the boundary condition, and F_i are so chosen that no additional stresses occur at the boundary. These requirements are not always simply met, especially if the contour is of arbitrary shape or if differing conditions exist on separate parts of the contour. A simple example is that of a rectangle of dimensions $2a$ and $2b$. When the origin is at the centroid, and the axis parallel to the sides of the rectangle, a suitable form of the function is

$$(24) \quad F = F_0 + (x^2 - a^2)^2 (y^2 - b^2)^2 [\alpha_1 + \alpha_2 (x^2 + y^2) + \alpha_3 (x^2 y^2) + \dots]$$

if a symmetrical function is required. If for physical reasons it is known that a skew symmetrical function is desired, odd powers may be used in the bracket term.

In the Trefftz method, it is convenient to employ the same information as in the Ritz method, namely a knowledge of F_0 , if $\nabla^4 F_0 = 0$. In the case that F_0 is not a biharmonic function, usually one can construct a function F_0^1 satisfying $\nabla^4 F_0^1 = 0$, which will provide for the existence of mean stresses or of a linear distribution of stresses, which is known as an approximation of the real solution. The stress function is then constructed in the form

$$(25) \quad F = F_0^1 + F_1$$

in which the additional stress function F_1 provides the corrected stresses and the problem becomes a problem of approximating for F_1 by Trefftz's

method. To satisfy the requirement of (17) and (18), suitable forms of F_1 are

$$(26) \quad \begin{aligned} F_1 &= (x^2 + y^2) \sum \alpha_i \Phi_i(x \pm iy) \\ F_1 &= x \sum \alpha_i \Phi_i(x \pm iy) \\ F_1 &= y \sum \alpha_i \Phi_i(x \pm iy). \end{aligned}$$

Since it is possible to approximate to any "well-behaved" function in a uniformly convergent manner by harmonic polynomials[13], it becomes apparent from (26) that

$$\lim_{i \rightarrow \infty} (F_0^i + F_1) = F$$

and

$$\lim_{i \rightarrow \infty} I[F_0^i + F_1] = I[F].$$

This insures that the function (25) as well as its derivatives is represented by the approximation process in a uniformly convergent manner and hence in the limit will attain the prescribed values not only in the region R but on its contour Γ .

Similar consideration for the Ritz method follows from theorems by Weierstrass for the approximation by power series, and by Fourier for trigonometric series, and by still more general theorems[14] on the representation of functions by complete sets of orthogonal functions.

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NOTES ON THE HABITS OF JUNE BEETLES IN IOWA (PHYLLOPHAGA - COLEOPTERA)¹

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For many years Iowa farmers have been writing for information concerning the habits and control of June beetles and their grubs. The answers for these questions have been taken largely from the writings of workers in other states, since little attention has been given in Iowa to the habits of the adult beetles. The present report is based upon experimental work done in various parts of the state during the spring and summer of 1932. It includes observations on evening and morning flights of beetles, notes on copulation, and results of some light trap studies. Collection data are presented on the following species: *Phyllophaga hirticula* (Knoch), *P. tristis* (Fab.), *P. futilis* (Lec.), *P. fusca* (Froel.), *P. rugosa* (Mels.), *P. hornii* (Sm.), *P. inversa* (Horn), *P. vehemens* (Horn), *P. implicita* (Horn), *P. ilicis* (Knoch), *P. marginalis* (Lec.), *P. micans* (Knoch), *P. congrua* (Lec.), *P. crenulata* (Froel.), *P. prunina* (Lec.), *P. drakii* (Kby), *P. nitida* (Lec.), *P. fosteri* (Burm.), *P. balia* (Say), *P. fraterna* Harris, and *P. vilifrons* Lec.

EVENING AND MORNING FLIGHTS OF BEETLES

With a few exceptions [*P. farcta* (Lec.), *P. cribrata* (Lec.), *P. lanceolata* (Say), and *P. epigaea* (Wickh.)] *Phyllophaga* adults are typically nocturnal in habits. During the day the beetles may be found in the soil, under rubbish, logs, and boards. Early in the evening the adults leave these places, migrate to food plants, feed and mate, and with the coming of daylight they return to secluded places.

The literature is somewhat indefinite relevant to evening and morning periods of flight. S. H. Linton (1888) gave one of the early records concerning the time of flight of June beetles. He observed *P. tristis* flying from sundown until dark. E. A. Schwartz (1891) mentions that during the last week of April, at Washington, the evening flight did not start before 8 o'clock and not before 8:30 a fortnight later, whereas, at Plum Creek, Nebraska, this author noted flights of *P. crassissima* (Blanch.) occurring about one hour earlier than the evening flights at Washington. Schwartz observed *P. aemula* (Horn) flying after dark during the early part of March, and in the alpine regions of Utah he saw an undetermined species flying at 6:30 p.m. (9,000 feet altitude); later the same species was noted in flight half an hour earlier at an altitude of 11,000 feet. Forbes (1907) has given the best report of the evening and morning flights of June beetles.

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This writer found that flights were completed within a period of 15 to 20 minutes, the starting time depending upon the clearness of the sky. He found that the evening flight began from a few minutes before to a few minutes after 8 p.m., and the return flight in the morning began between 3:18 and 4:25 a.m. In 1909 Manee reported *P. tristis* flying from 6 to 8 p.m. in North Carolina. Langston (1929) noted *P. davisii* Langs. in the air one hour before dark.

The daily activities (emergence in the evening and the return to the soil in the morning) have been studied at four points in Iowa: (1) Leon, Iowa (extreme south-central), (2) Ames, Iowa (central), (3) Hampton, Iowa (north-central), (4) Marquette, Iowa (extreme northeastern). The time of these flights (table 1) agree with the observations reported by Forbes (1907) in Illinois. The criterion for the time as listed in table 1 is based upon the initial appearance of the beetles in flight.

TABLE 1. *Evening and morning flight times of Phyllophaga (Brood A, 1932)*

Locality	Date	Time of evening flight	Time of morning flight
Leon	5-19-32	8:07	3:45
	6-21-32	8:05	3:40
Ames	5-23-32	7:55	3:25
	6-20-32	7:59	3:31
Marquette	6-28-32	8:21	—
	7- 8-32	—	4:15
Hampton	5-12-32	8:04	4:00
	6- 1-32	8:00	4:09
	6-18-32	8:05	3:50
	6-22-32	8:01	3:59

In each of the localities the order of emergence of the different species remained the same, even though their starting times varied from 7:55 to 8:21 p.m. When numerous enough in a given locality to permit accurate observations, the species typically issued in the following order: *P. tristis*, *P. hornii*, *P. hirticula*, and *P. rugosa*. As a rule, *P. tristis* preceded the other species by about five minutes, after which *P. hornii*, *P. hirticula*, and *P. rugosa* followed each other at about two minute intervals.

The morning flights began from 3:25 to 4:15 a.m. Movements from the trees were accomplished either by (1) flying directly from the leaves and twigs, or (2) by loosening their holds and dropping to the ground. In the latter case, however, some of the beetles frequently took wing while falling and flew away, whereas, others made no apparent effort to fly and burrowed into the soil soon after striking the surface. On cool, cloudy mornings dropping to the ground was more prevalent than the flight method of departure. The different species departed from the trees in the same order and with the same relative differences in time in which they came.

Some records of evening emergence (flight) at Ames were obtained under cage conditions with *P. hirticula* and *P. hornii*. Morning records were not successful in the cages since the beetles did not remain on the leaves throughout the night. Invariably the evening emergence extended

over a period of approximately 50 minutes. The peak of emergence occurred 15 to 20 minutes after the first beetle issued from its hiding place. Twenty-five minutes after emergence was initiated, 71.4 per cent of *P. hirticula* and 71.2 per cent of *P. hornii* had departed from their hiding places. Records of evening flights for two species are given in table 2; the cage emergence data compare favorably with field observations.

TABLE 2. Evening emergence (flight) of *P. hirticula* and *P. hornii* (Brood A, 1932)

Minutes	Emerg'd (percentage)	
	<i>P. hirticula</i>	<i>P. hornii</i>
5	4.53	9.05
10	11.18	14.02
15	18.53	20.26
20	20.15	13.02
25	17.13	14.93
30	12.57	10.40
35	7.88	6.22
40	4.42	6.33
45	2.33	3.15
50	4.28	2.62
	100	100

At intervals of one minute the beetles were collected as they emerged from the soil in the cages. Later the sexes were determined for each collection and the male and female beetles were treated separately (fig. 1, B). It is interesting to note that the emergence curves for the sexes are quite similar, except that of the male apparently has a slight tendency to form a double peak of issuance. In the cage experiments the number of individuals of *P. hornii* were much less than in the tests with *P. hirticula* (see fig. 1, A). The peak of activity of the former seemed to be about five minutes before that of the latter, but flight in numbers extended over a longer time in the case of *P. hornii*.

COPULATION NOTES

The observations on copulation of June beetles include some unpublished records taken by R. L. Webster in Iowa. Some fairly abundant species were never seen in copula. Records of pairs found copulating in the field are listed in table 4, whereas table 3 gives complete records for three pairs of *P. hirticula* and four pairs of *P. tristis*. All pairs in coitu observed by the author were found on their food plants with one exception, *P. crenulata* being taken on grass under an oak tree.

TABLE 3. Length of copulation time of *Phyllophaga* (Brood A, 1932)

Species	Time started	Time ended
<i>P. hirticula</i>	8:46 p. m.	2:30 a. m.
"	8:42 "	2:33 "
"	8:51 "	11:45 p. m.
<i>P. tristis</i>	8:32 "	9:19 "
"	8:37 "	9:20 "
"	8:42 "	9:21 "
"	8:37 "	9:30 "

TABLE 4. *Copulation time of Phyllophaga*

Species	Time observed
<i>crenulata</i>	8:35 p. m.
"	8:41 "
"	8:30 "
<i>ilicis</i>	8:47 "
<i>anxia</i>	9:05* "
"	9:30* "
"	9:30* "
"	9:30* "
"	9:00* "
"	9:30* "
<i>rugosa</i>	10:50* "
"	10:35-10:40* "
"	8:35- 9:00* "
"	8:41 "
"	9:40* "
"	9:00- 9:28* "
"	9:41 "
"	8:53 "
"	10:37 "
"	11:01 "
<i>hirticula</i>	8:35 "
"	8:41 "
"	8:46 "
"	10:31 "
"	11:40 "
"	12:00 "
"	1:30 a. m.
"	2:00 "
"	9:53 p. m.
"	9:14 "
<i>nitida</i>	9:45* "
"	10:10-10:25* "
<i>balia</i>	10:10-10:25* "
<i>futilis</i>	8:30- 9:00* "
<i>implicata</i>	8:23 "
<i>prunina</i>	8:10 "

* Records of R. L. Webster

LIGHT TRAP STUDIES

Two light traps (gasoline lanterns being used as the source of light) were operated at Leon, Iowa, during the summer of 1932. Only 1,266 beetles were taken from these traps during the entire summer. These data do not compare favorably with the catches reported by Sanders and Fracker (1916) in Wisconsin and other workers.

Some writers have reported that females of most species of *Phyllophaga* are only slightly attracted to lights. In the case of *P. tristis* and *P. micans* the writer found that the females outnumbered the males. Of the total 1,266 beetles collected, 62.3 per cent were males and 37.7 per cent were females (see table 6).

COLLECTION RECORDS

Over 53,000 beetles were collected during 1932 in Iowa. The specimens are represented by 21 species, *P. congrua* being recorded for the first time in Iowa. Of this group, 14 species were collected on 18 different trees and shrubs, 14 species at light traps, and 15 from miscellaneous collections.

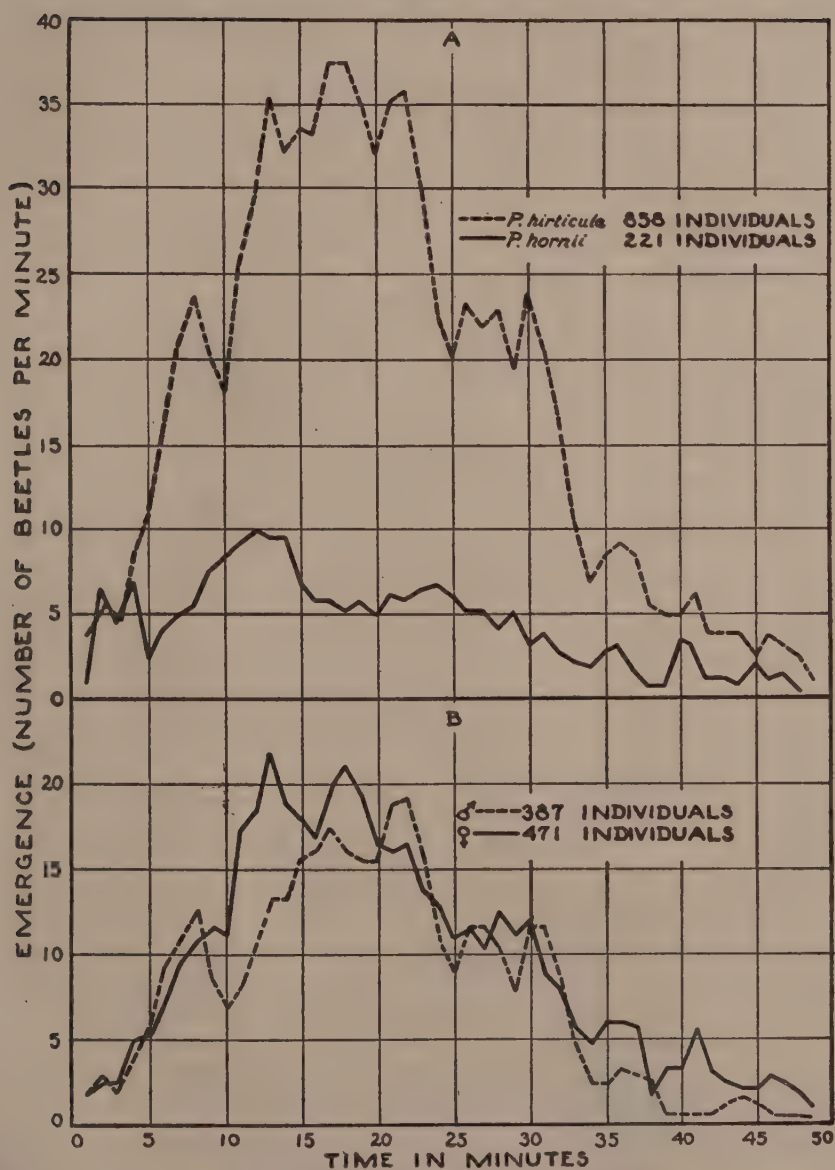


Fig. 1. A. Emergence of *P. hirticula* and *P. hornii*. B. Emergence of *P. hirticula* ♂♂ and ♀♀. (Three minute running average curves.)

Table 5 (continued)

<i>P. crenulata</i>	♂	11	1	3	1				2			1		3	1		1			5	29	34
<i>P. prunina</i>	♂		1																2	1		
<i>P. drakii</i>	♂												1						2	3		
<i>P. nitida</i>	♂																		2	3		
<i>P. fosteri</i>	♂																			3		
<i>P. balia</i>	♂																		2	2		
<i>P. fraternus</i>	♂																			1		
<i>P. nilifrons</i>	♂																			1		
Sub total	♂	18,163	1,417	677	334	255	125	126	48	44	15	19	47	20	5	2			789	524	22,807	
	♀	24,866	1,547	779	483	365	401	239	173	221	168	133	23	27	4	6	1	1	477	348	30,380	53,187
Total		43,029	2,964	1,456	817	620	526	434	299	269	212	148	137	70	47	9	8	3	1,266	872		53,187

TABLE 7. *Collection data by counties*
(Brood A, 1932)

Species	Counties									Total
	Franklin	Decatur	Jackson	Story	Clayton	Harrison	Monona	Dubuque	Iowa	
<i>P. hirticula</i>	32,782	3,359	1,178	136	16	100		52		37,623
<i>P. tristis</i>	10,501	9		110						10,620
<i>P. futilis</i>	137	57		764		108	129		1	1,196
<i>P. fusca</i>	990	69			51	26			1	1,137
<i>P. rugosa</i>	58	72	72		676	116	46	9	2	1,051
<i>P. hornii</i>		537				8				545
<i>P. inversa</i>	2	328				9				339
<i>P. vehemens</i>						145	80			225
<i>P. implicita</i>	4	26	2		99		32			163
<i>P. ilicis</i>		36	14		36			10		96
<i>P. marginalis</i>	5	48								53
<i>P. micans</i>		46								46
<i>P. congrua</i>						6	38			44
<i>P. crenulata</i>	34									34
<i>P. prunina</i>		3								3
<i>P. drakii</i>		2			1					3
<i>P. nitida</i>	3									3
<i>P. fosteri</i>		2								2
<i>P. balia</i>				2						2
<i>P. fraterna</i>		1								1
<i>P. vilifrons</i>					1					1
Total	44,516	4,595	1,266	1,012	880	518	325	71	4	53,187

The latter were taken under rubbish, behind the plow, while digging for white grubs, and the ones remaining were sent to the college for determination.

The numbers of individuals recorded in table 5 with the different host plants can not necessarily be considered significant in determining the food choice of the beetles, because more collections were made from some plants than others.

The number of females exceeded that of the males in some of the species. This was true in the case of the following beetles taken from host plants: *P. hirticula*, *P. fusca*, *P. rugosa*, *P. implicita*, and *P. crenulata*, while this was true only for *P. implicita* in the miscellaneous collections. The percentages of the total males and females taken from food plants and miscellaneous collections did not vary appreciably (table 6).

Although collections were made in only nine of the ninety-nine counties, they give a fair sample of the Phyllophaga occurring in the state, with the exception of the northwest corner (table 7).

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BACTERIA FERMENTING XYLAN¹

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Xylan is an important constituent of many of the so-called agricultural wastes which constitute a problem of economic utilization. It occurs abundantly in straws, cotton seed, and oat hulls, and makes up approximately one-third of the dried cornstalk. The development of a fermentation process for the commercial utilization of xylan would prove of great value to agriculture as well as to industry. With this purpose in mind, the present investigation is concerned with a systematic study of bacteria attacking purified xylan.

HISTORICAL

The literature dealing with the bacterial fermentation of pentosans is meager and generally has to do with the decomposition of plant tissue. No reference has come to the attention of the authors in which the action of known bacteria on purified xylan has been studied.

Swartz (1909) found that pentosans, mannans, and levulans were gradually decomposed by the aerobic bacteria of the alimentary tract, by soil organisms and by certain putrefactive forms. This decomposition took place with the formation of reducing substances.

Peterson, Fred and Verhulst (1921) in a study of corn silage fermentation, found that from 15 to 20 per cent of the pentosans disappeared. Verhulst, Peterson and Fred (1923) employed *Bacillus flavigena* (*Cellulomonas flavigena*) and *Bacillus coli communis* (*Escherichia coli*) in a medium of corn stover and yeast water to determine the distribution of pentosans in the corn plant. They concluded that *Bacillus flavigena* produced greater destruction of pentosan than did either *Bacillus coli communis* or an unnamed chromogenic form which had been isolated from green corn.

Gray and Chalmers (1924) found that *Microspira agarliquefaciens* digested cellulose much more rapidly in the presence of straw gum (pentosans), xylose, and arabinose.

Bottini (1925) concluded that the decomposition of pentosans in horse manure by microorganisms began early and that after eight months 16 per cent of the pentosans remained.

Patrick, Werkman and Hixon (1930) described the production of a pentose, probably xylose, from purified xylan by an actinomyces which did not attack xylose.

EXPERIMENTAL RESULTS

Cornstalks were ground to the fineness of sawdust and added to a 5 per cent solution of NaOH for twenty-four hours in order to dissolve the

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xylan. The pulp was separated from the liquid by means of a tincture-press. It was found by experience that one volume of alcohol added to one and one-half volumes of the xylan solution precipitated the maximum amount of xylan with a small percentage of lignin. The precipitate was filtered from the suspension, placed in a 5 per cent NaOH solution, and allowed to stand for several hours to remove by sedimentation any insoluble matter. The liquid was then filtered and alcohol added as before, to precipitate the xylan. This process was repeated several times. The residue was then made acid to litmus paper, alcohol was added to the gum to remove the water, and the suspension filtered. Ether was added to remove the alcohol; the excess ether was filtered off and the residue evaporated to dryness. Since the xylan was stirred often, it dried to a fine yellowish-brown powder. For the preparation of the xylan in part, the authors are indebted to Dr. R. M. Hixon of the Department of Chemistry.

Xylan was determined quantitatively according to "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists" (1925).

Analysis of the purified xylan was as follows:

Xylan	87.1	per cent
Ash	1.5	" "
Lignin	3.2	" "
Water	8.4	" "
	<hr/>	
	100	" "

A basal medium of xylan and ammonium chloride was prepared as follows:

Xylan	2.5 gm.
NH ₄ Cl	2.0 "
K ₂ HPO ₄	1.0 "
Water	1000.0 cc.
Adjusted to pH	7.0 and an indicator added.

The present work was limited to the use of a xylan-ammonium chloride agar medium for isolation of the organisms in the endeavor to insure utilization of xylan by the organisms as a source of carbon for energy.

There is no method known by which xylan can be prepared entirely free from lignin. Since media prepared with purified lignin have failed to show growth with a large number of organisms within a reasonable period of incubation, it is believed that the small percentage of lignin present did not influence the results materially.

Isolations were made and replated on the xylan medium solidified by addition of 1.5 per cent agar and only those organisms which produced acid or acid and gas were retained for study.

Production of acetoin was determined according to the method outlined by Werkman (1930) as well as by the 'standard' procedure. In most instances the former method gave a more rapid positive test and a more intense color than was present in the tubes with no added Fe Cl₃ solution.

Fermentation of aesculin is difficult to detect unless gas is produced and for this reason was confirmed by adding a few drops of a one per cent solution of ferric chloride to the fermented medium, when a red to brown coloration developed. Uninoculated tubes gave no change of color.

The production of hydrogen sulphide was determined in a medium prepared as follows: proteose-peptone, 20 grams; dipotassium phosphate, 0.3 grams; cysteine, 1.0 gram; ferrous chloride 1.0 gram; dextrose, 1 gram; agar, 5.0 grams; distilled water, 1 liter. The reaction should be between pH 6.8 and 7.4. The iron salt is sterilized separately or with the dextrose and added to the basal medium under aseptic conditions and the finished medium tubed in sterile test tubes. Ferrous salts are employed due to the somewhat greater solubility of the hydroxide and the instability of ferric sulphide. Organic iron salts may be used. The organic salts assist in colloidal dispersion of the iron salt and do not undergo as marked hydrolysis as do the inorganic salts. We have used the tartrate, acetate, citrate and lactate. These may be used in amounts equivalent in iron. Inoculate by means of a needle both on the surface and into the medium. The coloration is black and usually develops within twelve hours.

Morphological descriptions were made from young, stained cultures (18 hours) from glucose phosphate agar.

More than 250 isolations were made from soils, decaying wood, silage, and vegetables.

DESCRIPTIONS OF SPECIES

Species of the genus Bacillus fermenting xylan

A key to the species of the genus *Bacillus fermenting xylan* has been constructed.

KEY TO SPECIES

- A. Gelatin liquefied.
 - B. Acid in litmus milk. No curd. No peptonization.
 - C. Acid from inulin (No. 9).
 - Bacillus lignorum*
 - 2C. No acid from inulin (No. 11).
 - Bacillus picrogenes*
 - 2B. Acid and curd with no peptonization in litmus milk.
 - C. Nitrates reduced to nitrites.
 - D. Acid from inulin (No. 2).
 - Bacillus flexus*
 - 2D. No acid from inulin (No. 5).
 - Bacillus lignivorans*
 - 2C. Nitrates not reduced (No. 10).
 - Bacillus melonis*
- 2A. Gelatin not liquefied.
 - B. Acid from litmus milk. No curd. No peptonization.
 - C. Nitrates reduced.
 - D. Acid from xylose and galactose (No. 1).
 - Bacillus lautus*
 - 2D. No acid from xylose and galactose (No. 6).
 - Bacillus xylophagus*
 - 2C. Nitrates not reduced.
 - D. No acid from lactose (No. 4).
 - Bacillus acidifaciens*
 - 2D. Acid from lactose (No. 7).
 - Bacillus glutinis*
 - 2B. No change in litmus milk.

C. Acid from levulose and melezitose (No. 13).

—*Bacillus concoctans*

2C. No acid from levulose or melezitose (No. 3).

—*Bacillus xylanicus*

Bacillus lignorum sp. nov.

Culture 9; source, rotted apple wood.

Morphology. Twenty-four hour glucose-phosphate-peptone agar culture, 30° C. Rods, single or in pairs, 0.6–0.8 μ by 1.3–2.2 μ , ends rounded, spores present, centrally located, slightly larger than vegetative cell, motile (peritrichous flagella), Gram-positive and no iodophilic granules. Capsules absent in twenty-four hour litmus milk culture.

Cultural Characteristics. Agar streak: growth scanty, beaded, flat, dull, smooth, opaque, grayish-white, viscid, odor absent. Medium: unchanged after 3 days at 30° C.

Plain broth: no surface growth, slight cloudiness, no odor, no appreciable amount of sediment after 12 days at 30° C.

Litmus milk: acid after 10 days, reduction of litmus began on the fourth day. No coagulation and no peptonization within 10 days at 30° C.

Gelatin: growth best at top. Line of puncture, filiform, liquefaction saccate, beginning on seventh day. Medium uncolored after 30 days at 20° C.

Potato slant: growth, yellowish brown, shining, moderate. Medium darkened after 21 days at 30° C.

Biochemical Characters. Indol not formed. Acetyl-methyl-carbinol not formed from glucose. Nitrates reduced. Utilized ammonium chloride as source of nitrogen. Methyl red reaction negative. H₂S not produced. Gelatin liquefied. Diastase doubtful.

Fermentation Reactions. Acid and no gas from aesculin, amygdalin, arabinose, dextrin, galactose, glucose, inulin, maltose, melezitose, raffinose, salicin, starch, sucrose, trehalose, xylan and xylose. The fermentation of xylan was not vigorous.

No acid or gas from arabitol, dulcitol, erythritol, glycogen, inositol, lactose, levulose, pectin, rhamnose and sorbitol.

Bacillus picrogenes sp. nov.

Culture 11; source, decayed watermelon.

Morphology. Twenty-four hour glucose-phosphate-peptone agar culture, 30° C. Rods, single or in chains, 0.6–0.8 μ by 2.2–2.6 μ , ends rounded, central spores, motile (peritrichous flagella), Gram-positive, no iodophilic granules. Capsules absent in twenty-four hour litmus milk cultures.

Cultural Characteristics. Agar streak: growth scanty, beaded, raised, glistening smooth, translucent, gray, butyrous, odor absent. Medium: unchanged after 3 days at 30° C.

Plain broth: no surface growth, no appreciable amount of cloudiness or sediment after 12 days at 30° C.

Litmus milk: acid, reduction of litmus began on second day, completed in 10 days. No coagulation and no peptonization after 10 days at 30° C.

Gelatin: growth best at the top; line of puncture filiform; liquefaction napiform; began about fourth day. Medium uncolored after 30 days at 20° C.

Potato slant: growth dry, grayish tan, thick, wrinkled and dull after 21 days at 30° C.

Biochemical Characters. Indol not formed. Acetyl-methyl-erbinol not produced from glucose. Nitrates not reduced. Utilized ammonium chloride as a source of nitrogen. Methyl red reaction negative. H₂S not produced. Gelatin liquefied. Diastase not produced.

Fermentation Reactions. Acid and no gas from amygdalin, arabinose, dextrin, glucose, glycogen, lactose, levulose, melezitose, raffinose, salicin, starch, sucrose, trehalose, xylan and xylose.

No acid or gas from aesculin, arabitol, dulcitol, erythritol, galactose, inositol, inulin, maltose, pectin and rhamnose.

Bacillus flexus Batchelor 1919

Culture 2; source, decayed apple wood.

Morphology. Twenty-four hour glucose-phosphate-peptone agar culture, 30° C. Rods, single or in pairs, 0.7-1.0 μ by 2.1-3.8 μ , ends rounded, endospores present, sluggishly motile with peritrichous flagella, Gram-positive, iodophilic granules absent. Capsules absent in twenty-four hour litmus milk.

Cultural Characteristics. Agar streak: growth scanty, spreading, flat, glistening, smooth, translucent, grayish-white, viscid, odor absent. Medium: unchanged after 3 days at 30° C.

Plain broth: no surface growth, slight cloudiness, no odor, and no appreciable amount of sediment after 12 days at 30° C.

Gelatin: growth best at top. Line of puncture filiform. Liquefaction napiform, began after 4 days at 20° C. Culture was observed for 30 days.

Litmus milk: acid, coagulated.

Potato slant: growth, light yellow, thin. Medium: unchanged after 15 days at 30° C.

Biochemical Characters. Indol not formed. Acetyl-methyl-carbinol not produced from glucose. Nitrates reduced after 7 days. Utilized ammonium chloride as source of nitrogen. Methyl red reaction negative. H₂S produced. Gelatin liquefied. Diastase produced.

Fermentation Reactions. Acid but no gas from aesculin, amygdalin, arabinose, dextrin, galactose, glucose, glycogen, inulin, lactose, levulose, maltose, melezitose, raffinose, salicin, starch, sucrose, trehalose and xylan.

No acid or gas from arabitol, dulcitol, erythritol, inositol, pectin, rhamnose, sorbitol and xylose.

Bacillus melonis sp. nov.

Culture 5; source, decayed watermelon.

Morphology. Twenty-four hour glucose-phosphate-peptone agar culture, 30° C. Rods, single or in pairs, 0.7-0.9 μ by 2.7-3.2 μ , ends rounded, endospores present, subterminal, same size to slightly larger than diameter of vegetative cell, motile by means of peritrichous flagella, Gram-positive, no iodophilic granules. Capsules absent in twenty-four hour milk cultures.

Cultural Characteristics. Agar streak: growth moderate, filiform, flat, glistening, smooth, translucent, gray, odor absent. Medium slightly green after 3 days at 30° C.

Plain broth: no surface growth. Moderate cloudiness. Sediment flocculent and scanty after 12 days at 30° C.

Litmus milk: acid and coagulated after 7 days. Litmus reduced at the end of 10 days at 30° C.

Gelatin stab: growth best at surface, line of puncture filiform. Liquefaction napiform, began after 4 days. Medium: unchanged after 30 days at 20° C.

Potato slant: growth scant and glistening.

Biochemical Characters. Indol not formed. Acetyl-methyl-carbinol not produced from glucose. Nitrates not reduced. Utilized ammonium chloride as source of nitrogen. Methyl red reaction negative. H₂S produced slightly. Gelatin liquefied. Diastase produced.

Fermentation Reactions. Acid but no gas from arabinose, cellobiose, dextrin, glucose, lactose, levulose, maltose, melezitose, salicin, starch, sucrose, xylan and xylose.

Neither acid nor gas from aesculin, amygdalin, arabitol, dulcitol, erythritol, galactose, inulin, inositol, pectin, raffinose, rhamnose, sorbitol and trehalose.

Bacillus lignivorans sp. nov.

Culture 10; source, decayed maple wood.

Morphology. Twenty-four hour glucose-phosphate-peptone agar culture, 30° C. Rods, single or in pairs, 0.8-1.0 μ by 1.8-2.4 μ , ends rounded, endospores present, centrally located, slightly larger than vegetative cell, motile by means of peritrichous flagella, Gram-positive (young cultures), no iodophilic granules. Capsules absent in twenty-four hour culture of litmus milk.

Cultural Characteristics. Agar streak: growth moderate, spreading, glistening, smooth, translucent, yellowish, butyrous, medium unchanged. Odor absent after 3 days at 30° C.

Plain broth: pellicle on surface, slight cloudiness, odor absent. No appreciable amount of sediment after 12 days at 30° C.

Litmus milk: acid after 4 days, coagulated after 7 days, litmus was reduced after 10 days at 30° C.

Gelatin stab: best growth on surface. Line of puncture winged. Liquefaction napiform, began after 7 days. Medium: Color unchanged after 30 days at 20° C.

Potato slant: abundant brownish-yellow, lustrous growth. Medium browned after 21 days at 30° C.

Biochemical Characters. Indol not formed. Acetyl-methyl-carbinol not produced from glucose. Nitrates reduced. Utilized ammonium chloride as source of nitrogen. Methyl red reaction was negative. H₂S produced. Gelatin liquefied. Diastase produced.

Fermentation Reactions. Acid but no gas from amygdalin, arabinose, dextrin, galactose, glucose, glycogen, lactose, levulose, maltose, pectin, raffinose, salicin, starch, sucrose, trehalose, xylan and xylose.

No acid or gas from aesculin, arabitol, dulcitol, erythritol, inositol, inulin, melezitose, rhamnose and sorbitol.

Bacillus lautus Batchelor, 1919

Culture 1; source, decayed apple wood.

Morphology. Twenty-four hour glucose-phosphate-peptone agar culture 30° C. Rods, single or in chains, 0.7-0.8 μ by 4.0-4.5 μ , ends rounded and slightly pointed, endospores present, motile by peritrichous flagella, Gram-positive, no iodophilic granules. Capsules absent in litmus milk culture.

Cultural Characteristics. Agar streak: growth, scanty, spreading, flat, glistening, smooth, translucent, yellowish-gray, and butyrous. Odor absent. Medium unchanged after 3 days at 30° C.

Plain broth: no surface growth, moderate cloudiness, no odor, sediment flocculent and scanty after 12 days at 20° C.

Litmus milk: acid after 10 days at 30° C. No coagulation or peptonization.

Gelatin stab: growth best at top. Line of puncture filiform. No liquefaction. Medium: color unchanged after 30 days at 20° C.

Potato slant: growth yellowish-white, smooth, and lustrous. Medium: color unchanged after 21 days at 30° C.

Biochemical Characters. Indol not formed. Acetyl-methyl-carbinol not produced from glucose. Utilized ammonium chloride as source of nitrogen. Methyl red reaction was negative. H₂S not produced. Gelatin not liquefied. Diastase slightly produced.

Fermentation Reactions. Acid, but no gas from amygdalin, arabinose, cellobiose, dextrin, galactose, glucose, inulin, lactose, maltose, raffinose, salicin, starch, sucrose, trehalose, xylan and xylose.

Neither acid nor gas was formed from aesculin, arabitol, duleitol, erythritol, inositol, levulose, melezitose, rhamnose, and sorbitol.

Batchelor (1919) found that *Bacillus lautus* grown in litmus milk, produced acid with a rapid reduction of the litmus. After 10 days, culture number 1 showed only an acid reaction. Batchelor made no mention of the time the milk culture was allowed to incubate.

Bacillus acidifaciens sp. nov.

Culture 4; source, decayed maple wood.

Morphology. Twenty-four hour glucose-phosphate-peptone agar culture, 30° C. Rods, single or in pairs, 0.5-0.8 μ by 2.5-4.8 μ , ends rounded, endospores present, centrally located, much larger than vegetative cell giving clostridial shape, motile by peritrichous flagella, Gram-positive, no iodophilic granules. Capsules absent in litmus milk culture.

Cultural Characteristics. Agar streak: growth scanty, beaded, raised, glistening, smooth, translucent, gray and butyrous, odor absent, color of medium unchanged after 3 days at 30° C.

Plain broth: no surface growth, moderate cloudiness, odor absent. No appreciable sediment after 12 days at 30° C.

Litmus milk: slight acid after 7 days. Litmus reduced after 10 days. No coagulation or peptonization after 10 days at 30° C.

Gelatin stab: growth best at top, line of puncture filiform, no liquefaction, no change in medium after 30 days at 20° C.

Potato slant: growth scant, yellowish-brown and lustrous. Medium unchanged in appearance after 21 days at 30° C.

Biochemical Characters. Indol not produced. Acetyl-methyl-carbinol not produced from glucose. Nitrates not reduced. Utilized ammonium chloride as source of nitrogen. Methyl red reaction negative. H₂S was not produced. Gelatin not liquefied. Diastase produced.

Fermentation Reactions. Acid but no gas produced from arabinose, cellobiose, dextrin, glucose, inulin, levulose, maltose, salicin, starch, sucrose, trehalose, xylan (weak) and xylose.

No acid or gas from aesculin, amygdalin, duleitol, arabitol, erythritol,

galactose, inositol, lactose, melezitose, pectin, raffinose, rhamnose and sorbitol.

Bacillus xylophagus sp. nov.

Culture 6; source, decayed apple wood.

Morphology. Twenty-four hour glucose-phosphate-peptone agar culture, 30° C. Rods, single or in pairs, 0.6-0.9 μ by 2.3-3.4 μ , ends rounded, endospores present, centrally located, non-motile, Gram-positive (loses Gram-positive nature early), no iodophilic granules.

Cultural Characteristics. Agar streak: growth scanty, beaded, spreading, flat, glistening, smooth, translucent, yellowish-white and butyrous, odor absent. Medium unchanged after 3 days at 30° C.

Plain broth: no surface growth, slight cloudiness, no odor, no appreciable amount of sediment after 12 days at 30° C.

Potato slant: no growth after 21 days at 30° C.

Litmus milk: acid after 4 days. Coagulated after 10 days. No reduction of litmus. No peptonization after 10 days. Incubated at 30° C.

Gelatin stab: growth best at the top, line of puncture filiform, no liquefaction, medium unchanged after 30 days at 20° C.

Biochemical Characters. Indol not formed. Acetyl-methyl-carbinol not produced from glucose. Nitrates not reduced. Utilized ammonium chloride as source of nitrogen. H₂S not produced. Gelatin not liquefied. Diastase produced.

Fermentation Reactions. Acid but no gas from aesculin, amygdalin, dextrin, glucose, glycogen, inulin, lactose, levulose, maltose, melezitose, raffinose, salicin, starch, sucrose, trehalose and xylan (weak).

No acid or gas from arabinose, arabitol, cellobiose, dulcitol, erythritol, galactose, inositol, pectin, rhamnose, sorbitol and xylose.

Bacillus glutinis sp. nov.

Cultures 7 and 8; source, decayed apple wood.

Synonymy. Possibly synonymous with *B. fusus* Batchelor 1919.

Morphology. Twenty-four hour glucose-phosphate-peptone agar, 30° C. Rods, single or in chains, 0.7-0.9 μ by 1.8-3.1 μ , ends rounded, endospores located centrally to slightly off center, motile, Gram-positive, no granular appearance with iodine. Capsules absent in litmus milk cultures.

Cultural Characteristics. Agar streak: growth scanty, beaded, raised, glistening, smooth, translucent, gray, and viscid. Odor absent. Medium unchanged after 3 days at 30° C.

Plain broth: no surface growth, slight cloudiness, odor absent, no appreciable amount of sediment after 12 days at 30° C.

Litmus milk: acid after 2 days. Coagulated after 7 days. Reduction of litmus began after 4 days. No peptonization within 10 days at 30° C. Culture 8 showed peptonization.

Gelatin stab: best growth at the surface, line of puncture winged, no liquefaction, no change in medium after 30 days at 20° C.

Potato slant: growth moderate, smooth, and brown. Medium browned after 21 days at 30° C.

Biochemical Characters. Indol not formed. Acetyl-methyl-carbinol not produced from glucose. Nitrates slightly reduced after 5 to 7 days to nitrites. Ammonium chloride utilized as a source of nitrogen. Methyl

red reaction negative. H_2S not produced. Gelatin not liquefied. Diastase produced.

Fermentation Reactions. Acid but no gas from aesculin, amygdalin, arabinose, cellobiose, dextrin, galactose, glucose, inulin, lactose, levulose, maltose, raffinose, salicin, starch, sucrose, trehalose, xylan and xylose.

No acid or gas from arabitol, dulcitol, erythritol, inositol, melezitose, pectin, rhamnose and sorbitol.

Bacillus xylanicus sp. nov.

Culture 3; source, decayed apple wood.

Morphology. Twenty-four hour glucose-phosphate-peptone agar, 30° C. Rods, single or in pairs, 0.8-1.0 μ by 2.0-3.5 μ , ends rounded, endospores present, caused swelling of vegetative cell, located centrally to slightly off center, give snow-shoe appearance to organism, Gram-positive. Capsules absent in litmus milk culture.

Cultural Characteristics. Agar streak: growth scant, beaded, flat, dull, smooth, opaque, gray-white and viscid, odor absent. Medium browned after 3 days at 30° C.

Plain broth: ring surface growth, slight cloudiness, no odor, and no appreciable amount of sediment after 12 days at 30° C.

Litmus milk: milk unchanged after 10 days at 30° C.

Gelatin stab: growth best at surface. Line of puncture filiform. No liquefaction. Medium unchanged after 30 days at 20° C.

Potato slant: thin, cream colored, lustrous growth. Medium unchanged after 21 days at 30° C.

Biochemical Characters. Indol not formed. Acetyl-methyl-carbinol not produced from glucose. Nitrates slightly reduced on fifth and seventh days. Utilized ammonium chloride as a source of nitrogen. Methyl red reaction was negative. H_2S was not produced. Gelatin was not liquefied. Diastase production was doubtful.

Fermentation Reactions. Acid but no gas was produced from aesculin, amygdalin, arabinose, cellobiose, dextrin, galactose, glucose, inulin, lactose, maltose, raffinose, salicin, starch, sucrose, trehalose, xylan or xylose.

Neither acid nor gas from arabitol, dulcitol, erythritol, inositol, levulose (?), melezitose, pectin, rhamnose or sorbitol.

Bacillus concoctans sp. nov.

Culture 13; source, soil.

Morphology. Twenty-four hour glucose-phosphate-peptone agar, 30° C. Rods, single or in chains, 0.8-1.0 μ by 1.3-1.8 μ , ends rounded, endospores present, located centrally to slightly off center, do not markedly swell vegetative cell, motile by peritrichous flagella, Gram-positive with no iodophilic granules. Capsules absent in litmus milk culture.

Cultural Characteristics. Agar streak: growth scanty, spreading, flat, glistening, translucent, white and viscid. No odor. Medium unchanged after 3 days at 30° C.

Plain broth: no surface growth, no cloudiness. Odor absent. Sediment flocculent and abundant after 12 days at 30° C.

Litmus milk: unchanged after 10 days at 30° C.

Gelatin stab: growth scant at surface. Line of puncture filiform. No liquefaction. No change in medium after 30 days at 20° C.

Potato slant: growth wrinkled, dull and gray. Medium darkened after 21 days at 30° C.

Biochemical Characters. Indol not formed. Acetyl-methyl-carbinol not produced from glucose. Nitrates reduced after 7 days. Utilized ammonium chloride as a source of nitrogen. Methyl red reaction was negative. H₂S was not produced. Gelatin was not liquefied. Diastase produced.

Fermentation Reactions. Acid but no gas from aesculin, amygdalin, arabinose, dextrin, glucose, lactose, levulose, maltose, melezitose, raffinose, salicin, starch, sucrose, xylan (weak) and xylose.

No acid or gas from arabitol, dulcitol, erythritol, galactose, inositol, pectin, rhamnose and sorbitol.

Species of the genus Achromobacter

Two organisms belonging to the genus *Achromobacter* were found to dissimilate xylan with the production of acid.

Achromobacter arabinosaceum sp. nov.

Culture 16; source, soil.

Morphology. Twenty-four hour glucose-phosphate-peptone agar, 30° C. Short rods, 0.6-0.8 μ by 1.1-1.5 μ , ends rounded, endospores none, non-motile, Gram-negative, iodophilic granules present. Capsules not observed in litmus milk although slime was produced in less than 24 hours.

Cultural Characteristics. Agar streak: growth moderate, filiform, raised, glistening, smooth, translucent, yellow-gray, butyrous, odor absent. Medium unchanged after 3 days at 30° C.

Plain broth: no surface growth, moderate cloudiness, no odor, no appreciable amount of sediment after 12 days at 30° C.

Litmus milk: acid after 4 days. Coagulated after 7 days.

Litmus reduced before 10 days at 30° C.

Gelatin stab: best growth at the surface. Line of puncture filiform. Liquefaction crateriform beginning within 4 days. Medium uncolored after 10 days at 20° C.

Potato slant: growth yellow-brown, lustrous and elevated. Medium darkened after 21 days at 30° C.

Biochemical Characters. Indol not formed. Acetyl-methyl-carbinol not produced from glucose. Nitrates not reduced. Utilized ammonium chloride as source of nitrogen. Methyl red reaction was positive. H₂S produced. Gelatin liquefied. Diastase produced.

Fermentation Reactions. Acid but no gas from amygdalin, arabinose, dextrin, glucose, glycogen, inulin, lactose, salicin, starch, sucrose, xylan and xylose.

No acid or gas from aesculin, dulcitol, erythritol, galactose, glycogen, inositol, levulose, maltose, melezitose, pectin, raffinose, rhamnose, sorbitol and trehalose.

Achromobacter dianthi sp. nov.

Culture 24; source, decayed watermelon.

Morphology. Twenty-four hour glucose-phosphate-peptone agar, 30° C. Rods, single or in chains, 0.8-1.0 μ by 1.9-4.0 μ , ends rounded, endospores absent, motile by peritrichous flagella, Gram-positive, no iodophilic granules. Capsules present in 24 hour milk culture.

Cultural Characteristics. Agar streak: growth moderate, filiform effuse, glistening, smooth, opaque, flesh pink and viscid. Odor absent. Medium unchanged after 3 days at 30° C.

Plain broth: thin, easily-broken pellicle, slight cloudiness, sediment compact and scanty, odor absent after 12 days at 30° C.

Litmus milk: acid after 7 days. Reduction of litmus began after 2 days and was complete after 10 days. No peptonization within 10 days at 30° C.

Gelatin stab: growth best at surface. Line of puncture, filiform. Liquefaction began after 4 days, napiform. Medium not colored within 30 days at 20° C.

Potato slant: growth abundant, elevated, brownish- yellow, and rough. Medium browned within 21 days at 30° C.

Biochemical Characters. Indol not formed. Acetyl-methyl-carbinol not produced from glucose. Nitrates reduced. Utilized ammonium chloride as source of nitrogen. Methyl red reaction was negative. H₂S was produced. Gelatin was liquefied. Diastase was produced.

Fermentation Reactions. Acid but no gas from amygdalin, arabinose, dextrin, glucose, lactose, levulose, maltose, melezitose, raffinose, salicin, starch, sucrose, trehalose, xylan and xylose.

Neither acid nor gas from aesculin, arabitol, dulcitol, erythritol, galactose, glycogen, inositol, inulin, pectin, rhamnose and sorbitol.

SUMMARY AND CONCLUSIONS

Bacteria capable of fermenting xylan, are widely distributed in nature. Xylan is relatively resistant to bacterial attack when compared with the simple carbohydrates. Of the cultures found to attack the pentosan with the production of acid, not one produced gas as indicated by the ordinary fermentation tube method. The quantity of acid produced was never great.

Several new species of bacteria have been described, nine in the genus *Bacillus* and two in the genus *Achromobacter*. These studies are being continued with the anaerobic organisms and on products of fermentation of xylan. Difficulty in obtaining chemically pure xylan in addition to its relative resistance to bacterial attack, makes it unsuitable for use in systematic studies for purposes of differentiation.

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THE INSECTICIDAL ACTION OF SOME FURAN COMPOUNDS

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INTRODUCTION

Apparently it is not possible at the present stage of development to formulate any rigorous rules correlating chemical constitution with insecticidal action, and in agreement with such inadequate knowledge it is extremely difficult to predict with confidence the insecticidal action of new organic compounds. This condition is not peculiar to studies on insecticidal action, for the same difficulty is patent, in varying degrees, with the many comprehensive studies reported on the correlation of chemical constitution with physiological action. In short, we are still at the empirical stage, and a rather crude empirical stage in many respects, in correlating chemical constitution with physiological action on practically all types of living things.

Despite the fact that furfural, the most important furan compound, was discovered one hundred years ago, it is only in recent years that certain new furan types (particularly those having nuclear substituents) have become available. Some of these compounds, selected at random from our studies on furan compounds, have been examined with a view to determining their possible insecticidal value. The results of this preliminary study are now reported, and although a large number of the compounds investigated are of relatively little value, some appear to be highly effective and indicate avenues of approach for the synthesis of compounds which it is hoped will prove of general value. In addition, of course, it is to be expected that as the field is explored more deeply some significant generalizations might ensue which will assist in formulating more exact correlations between chemical constitution and insecticidal action.

The problem, as it involves furan compounds, has recently assumed a position of some importance as a consequence of the elegant studies by La Forge, Haller, Clark, Smith, Butenandt, Robertson, Takei [See, Browne, *Science*, 76, 454 (1932)] and others on the constitution of rotenone. Rotenone has some characteristics of an ideal insecticide because of its high toxicity towards insects and its apparent harmlessness to animals. Rotenone is a nitrogen-free compound containing a furanic nucleus. It would be presumptuous to ascribe the valuable properties of rotenone to the furanic nucleus. Notwithstanding, it seems altogether reasonable to expect that there might emerge from a study of substituted furans or hydrogenated furans, patterned after fragments of the rotenone molecule, insecticides of economic value.

EXPERIMENTAL PART

Insecticidal Action.—The preliminary studies on the insecticidal action of the compounds examined in this investigation were carried out in ac-

cordance with the method of Peet and Grady.^{1a} About one hundred flies, raised under optimal conditions, and whose resistance to the spraying solvent or medium was previously determined, were placed in the test-chamber. No flies were used in which the kerosene check showed over 5 per cent to be brought down, and more usually none came down in the initiatory experiments.

The test-chamber was a cubical room with a six-foot edge, provided with windows on two sides, well lighted and previously well aerated. Twelve cc. of the solution to be tested was sprayed uniformly in the top of the chamber, through five port-holes one foot from the ceiling, from a standard atomizer at twelve pounds pressure. Exactly ten minutes after spraying, the inactive flies on the floor were counted from the windows. Another count was made at the end of thirty minutes, at which time the chamber was opened and the inactive flies collected and placed in cages with food and water, and then observed at the end of twenty-four hours.

Under these conditions a good pyrethrum spray will "down" or inactivate all of the flies in less than ten minutes, and at the end of twenty-four hours from 20 per cent to 30 per cent of those that were inactivated will have recovered.

The results are given in Table 1. The saturated solutions mentioned there contained less than 2 per cent. The concentration given refers to grams of compound per 100 cc. of solution: i.e., 2 per cent is equal to 2 g. per 100 cc. The solvent key is as follows: K = kerosene ("Rayolite," a water-white, refined kerosene); H = cyclohexanone; N = high flash naphtha; Z = cellosolve. The superscript numerals refer to composition of solvents by volume: that is, K⁶⁰H⁴⁰ means 60 cc. of kerosene + 40 cc. of cyclohexanone.

The results given in Table 1 are the averages of three or more tests on each compound.

CHEMICAL.—*Monomethyl Ether of 2,4-Dihydroxybenzofuran* (Compound 3, table 1).—This compound was prepared [incidental to the proof of structure of 2,4-dihydroxybenzoyl-furan^{1b}] by two reactions: (1) from 2,4-dihydroxybenzoyl-furan, sodium hydroxide and dimethyl sulfate; and (2) from 1,3-dimethoxybenzene, furoyl chloride and aluminum chloride. The melting point and mixed melting point range of the compounds is 75-82° when crystallized from petroleum ether (b.p., 40-60°). The compound (or mixture of compounds) is soluble in sodium hydroxide, and for the particular purposes in hand no effort was made to establish the position of the methoxyl group,—particularly because of the low insecticidal value.

Furyl-1-pentene-1-one-3 (Compound 5), furyl-1-methyl-4-pentene-1-one-3 (Compound 6), and furyl-1-methyl-2-pentene-1-one-3 (Compound 8) were prepared in accordance with the directions of Kasiwagi.² His yields of the first two compounds were checked. No yield is given of the third compound of which we obtained 60 per cent.

^{1a} Peet and Grady, *J. Econ. Ent.*, **21**, 612 (1928).

^{1b} Gilman and Dickey, *Rec. trav. chim.*, **52**, 389 (1933).

² Kasiwagi, *Bull. Chem. Soc. Japan*, **1**, 90 (1926) [*C. A.*, **20**, 3004 (1926)].

The authors are grateful to George F. Wright for the 5-nitrofurfural^{3a} (Compound 1), 5-bromofurfural^{3b} (Compound 2), 5-bromofurylacrylic acid (Compound 10), 5-bromofuryl-bromoaerylic acid^{3c} (Compound 11), ethyl 5-bromofuryl-dibromopropionate^{3d} (Compound 29) and 5-bromofuryl methyl ketone^{3e} (Compound 32).

Tetrahydrofurfuryl acetate (Compound 12) and tetrahydrofurfuryl benzoate (Compound 14) were prepared after the method of Zanetti.⁴

Tetrahydrofurfuryl trimethylacetate (Compound 13) was prepared in a customary manner from trimethylacetyl chloride and tetrahydrofurfuryl alcohol. The yield of ester distilling at 137-138°/40.5 mm. was 79 per cent; sp.g.₂₀²⁰, 0.9764. The compound has a pleasant odor.

Anal. Calcd: C, 64.46; H, 9.75. Found: C, 64.36 and 64.12; H, 9.80 and 9.53.

The authors are grateful to E. A. Zoellner for the trimethylacetic acid.

Phenyl furoate⁵ and ¹ (Compound 15) and *p*-hydroxyphenyl furoate¹ (Compound 16) have been described. Our phenyl furoate melted at 42° and was analyzed for C and H and also hydrolyzed to give furoic acid.

m-Cresyl furoate (Compound 17) was prepared in 90 per cent yield from equimolecular parts of *m*-cresol, furoyl chloride and sodium hydroxide. It melts at 39.5° and distils at 155°/5 mm. Hydrolysis gave furoic acid.

Anal. Calcd. for C₁₂H₁₀O₃: C, 71.28 and H, 4.95. Found: C, 70.95 and 70.62; H, 4.96 and 4.90.

Guaiacol furoate (Compound 18) was prepared like *m*-cresyl furoate (starting with guaiacol) in an 82.5 per cent yield. It melts at 76° and distils at 175°/5 mm. The saponification equivalent was 225 (calc. 218), and the furoic acid so obtained was identified by the method of mixed melting points.

Anal. Calcd. for C₁₂H₁₀O₄: C, 66.05; H, 4.59. Found: C, 65.92 and 66.19; H, 4.74 and 4.78.

p-Cresyl furoate (Compound 19), prepared like the preceding esters, melts at 55° and distils at 152°/5 mm.

Anal. Calcd. for C₁₂H₁₀O₃: C, 71.28; H, 4.95. Found: C, 70.83 and 71.66; H, 4.95 and 4.82.

Resorcinol difuroate (Compound 20) was prepared from 0.1 mole resorcinol, 0.2 mole furoyl chloride and 0.2 mole of potassium hydroxide. Some of the same ester was obtained in attempts to prepare the half-ester by using but one equivalent of furoyl chloride. The melting point is 130°.

³ (a) Gilman and Wright, *J. Am. Chem. Soc.*, 52, 4165 (1930); (b) Gilman and Wright, *ibid.*, 52, 1170 (1930); (c) Gibson and Kahnweiler, *Am. Chem. J.*, 12, 314 (1890); (d) Gilman and Wright, *J. Am. Chem. Soc.*, 52, 3349 (1930); (e) Gilman, Hewlett and Wright, *ibid.*, 53, 4192 (1931).

⁴ Zanetti, *J. Am. Chem. Soc.* 50, 1821 (1928).

TABLE 1. *Insecticidal action of some furan compounds*

No.	Compound	Solvent	Conc.	Downs			
				10 min.	30 min.	24 hours	
1.	5-Nitrofurfural, 5-NO ₂ C ₄ H ₃ OCHO	K ₂ CO ₃ N ⁴⁰	Sat'd	2	13
2.	5-Bromofurfural, 5-BrC ₄ H ₃ OCHO	K	Sat'd	9	41	26	
3.	Monomethyl Ether of 2, 4-Dihydroxybenzofuran, C ₆ H ₃ (OH)(OCH ₃)OCOC ₂ H ₅ O	K	Sat'd	8	37
4.	Furfuralacetophenone, C ₆ H ₃ OCH=CHCOC ₆ H ₅	K	2%	37	79	60	
5.	Furyl-1-pentene-1-one-3, C ₄ H ₃ OCH=CHCOC ₂ H ₅	K	2%	11	32
6.	Furyl-1-methyl-4-pentene-1-one-3, C ₄ H ₃ OCH=CHCOC ₂ H ₅ (CH ₃) ₂	K	2%	20	35
7.	2, 4-Dihydroxy-benzofuran, C ₆ H ₃ (OH) ₂ COC ₂ H ₅ O	K	1%	3	15
8.	Furyl-1-methyl-2-pentenone-3, C ₄ H ₃ OCH=CH(CH ₃)COC ₂ H ₅	K	2%	5	23
9.	Benzoyl Furan, C ₆ H ₅ COC ₂ H ₅ O	K ₂ CO ₃ N ²⁰	4%	60	85	58	
10.	5-Bromofurylacrylic Acid, BrC ₄ H ₂ OCH=CHCO ₂ H	K ₂ CO ₃ H ⁴⁰	2%	38	76	42	
11.	5-Bromofuryl-bromoacrylic Acid, BrC ₄ H ₂ OCH=CHBrCO ₂ H	K ₂ CO ₃ H ²⁰	2%	3	22	15	
12.	Tetrahydrofurfuryl Acetate, C ₄ H ₇ OCH ₂ OCOCH ₃	K	2%	6	8
13.	Tetrahydrofurfuryl Trimethylacetate, C ₄ H ₇ OCH ₂ OCO(CH ₃) ₃	K	2%	7	23	13	
14.	Tetrahydrofurfuryl Benzoate, C ₄ H ₇ OCO ₂ C ₆ H ₅	K	2%	5	21
15.	Phenyl Furoate, C ₆ H ₅ OCO ₂ C ₄ H ₃ OH	K ₂ CO ₃ N ¹⁰	2%	42	57	28	
16.	<i>p</i> -Hydroxyphenyl Furoate, C ₆ H ₄ OCO ₂ C ₄ H ₃ OH	K ₂ CO ₃ N ⁴⁰	Sat'd	4	23
17.	<i>m</i> -Cresyl Furoate, C ₆ H ₃ OCO ₂ C ₄ H ₃ OH	K	2%	12	31

Table 1, continued

18.	Guaiacol Furoate, $C_6H_4OCOC_6H_4OCH_3$	$K^{80}H^{20}$	2%	15	52	17
19.	<i>p</i> -Cresyl Furoate, $C_6H_3OCOC_6H_4CH_3$	$K^{80}N^{20}$	2%	67	81	61
20.	Resorcinol Difuroate, $[C_6H_4OCOC_6H_4]_2C_6H_3$	$K^{60}N^{40}$	Sat'd	10	31	...
21.	Hydroquinone Difuroate, $[C_6H_3OCOC_6H_4]_2C_6H_4$	$K^{60}N^{40}$	Sat'd	2	19	...
22.	Phenyl Furfurylate, $C_6H_5OCH=CHCO_2C_6H_4$	K	2%	62	82	37
23.	Guaiacol Furfurylate, $C_6H_3OCH=CHCO_2C_6H_4OCH_3$	$K^{60}N^{40}$	2%	6	39	...
24.	<i>p</i> -Cresyl Furfurylate, $C_6H_3OCH=CHCO_2C_6H_4CH_3$	K	2%	48	59	48
25.	<i>m</i> -Cresyl Furfurylate, $C_6H_3OCH=CHCO_2C_6H_4CH_3$	K	Sat'd	12	41	20
26.	<i>p</i> -Hydroxyphenyl Furfurylate, $C_6H_3OCH=CHCO_2C_6H_4OH$	$K^{60}N^{40}$	Sat'd	1	5	...
27.	Resorcinol Difurfurylate, $[C_6H_3OCH=CHCO_2]_2C_6H_4$	$K^{60}H^{40}$	2%	3	17	...
28.	Hydroquinone Dicinamate, $[C_6H_3CH=CHCO_2]_2C_6H_4$	$K^{60}N^{40}$	Sat'd	7	24	7
29.	Ethyl Bromofuryl-dibromopropionate, $BrC_4H_2OCHBrCHBrCO_2C_2H_5$	$K^{60}N^{40}$	Sat'd	0	16	...
30.	Trifurfurylamine, $[C_6H_4OCH_2]_3N$	K	Sat'd	9	35	14
31.	Ethyl Phenylsulfinate, $C_6H_5SO_2C_2H_5$	K	2%	6	30	...
32.	5-Bromofuryl Methyl Ketone, $BrC_4H_2OCOCH_3$	Z	2%	3	12	...

and that previously reported⁵ is 128-129°. The saponification equivalent (147) shows the compound to be a di-ester.

Anal. Calcd. for $C_{16}H_{10}O_6$: C, 64.43; H, 3.35. Found: C, 64.10, H, 3.19.

Hydroquinone difuroate (Compound 21). The ester was obtained in a 27 per cent yield by heating a mixture of 0.2 mole furoyl chloride, and 0.1 mole hydroquinone just below the boiling point of furoyl chloride (176°) until the mass solidified. After extraction with benzene and then with alcohol the compound was crystallized from acetone and was found to melt at 200°. The saponification equivalent was 150 (calcd. 149) and the products of hydrolysis were identified by mixed melting points.

Anal. Calcd. for $C_{16}H_{10}O_6$: C, 64.43; H, 3.35. Found: C, 64.59; H, 3.81.

Phenyl furylacrylate (Compound 22) was prepared in two ways: (1) from furylacryloyl chloride, phenol and sodium hydroxide and (2) by heating furylacryloyl chloride with phenol in benzene. The latter method gave a yield of 84 per cent and is recommended. The ester distills at 185°/4 mm.

Anal. Calcd. for $C_{13}H_{10}O_3$: C, 72.88; H, 4.67. Found: C, 72.36 and 72.78; H, 5.02 and 4.87.

Guaiacol furylacrylate (Compound 23) was prepared after the two methods described for the preceding ester, and again the latter method is recommended. The yield of ester distilling at 210°/6 mm. and melting at 105° was 70 per cent.

Anal. Calcd. for $C_{14}H_{12}O_4$: C, 68.85; H, 4.92. Found: C, 69.23 and 68.29; H, 4.70 and 5.12.

p-Cresyl furylacrylate (Compound 24) was prepared by refluxing 0.1 mole of furylacryloyl chloride and 0.1 mole of *p*-cresol in 200 cc. of benzene for two hours. The yield of ester melting at 75° and distilling at 195°/6 mm. was 71 per cent. The saponification equivalent was 213 (calcd. 212).

Anal. Calcd. for $C_{14}H_{12}O_2$: C, 73.68; H, 5.26. Found: C, 72.88 and 73.56; H, 5.31 and 5.51.

m-Cresyl furylacrylate (Compound 25) was prepared after the method described for the preceding ester. It distills at 185°/5 mm; d_4^{34} , 1.0728; n_D^{34} , 1.5980.

Anal. Calcd. for $C_{14}H_{12}O_3$: C, 73.68; H, 5.26. Found: C, 73.96 and 74.00; H, 5.45 and 5.39.

p-Hydroxyphenyl furylacrylate (Compound 26) was obtained in a 30 per cent yield by refluxing furylacryloyl chloride and hydroquinone in benzene. It melts at 173°. The same compound was obtained by using one or two equivalents of the acid chloride.

Anal. Calcd. for $C_{13}H_{10}O_4$: C, 67.83; H, 4.35. Found: C, 67.50 and 68.32; H, 4.34 and 4.19.

⁵ Baum, *Ber.* 37, 2931 (1904).

Resorcinol difurylacrylate (Compound 27) was prepared from the acid chloride and resorcinol. It melts at 112°.

Anal. Calcd. for $C_{20}H_{14}O_6$: C, 68.57; H, 4.00. Found: C, 69.23 and 69.02; H, 4.25 and 4.23.

Hydroquinone dicinnamate (Compound 28) melts at 189° which is in agreement with that previously given by Einhorn.⁶ Trifurfurylamine⁷ (Compound 30) and ethyl phenylsulfinate⁸ (Compound 31) have been described.

The following esters, some of which are closely related to those just described, were prepared incidental to another study on the antiseptic action of furan compounds as well as their action as fumigants. The results of these tests will be reported later.

Catechol difuroate, $o-(C_4H_3OCOO)_2C_6H_4$, was prepared by heating at 100° a mixture of 0.2 mole of furoyl chloride and 0.1 mole of catechol. The compound melts at 116° when crystallized from benzene and then from alcohol.

Anal. Calcd. for $C_{16}H_{10}O_6$: C, 64.43; H, 3.35. Found: C, 64.73; H, 3.81.

m-Hydroxyphenyl furylacrylate, $C_4H_3OCH=CHCOOC_6H_4OH(m)$, was prepared by refluxing 0.1 mole of furylacryloyl chloride and 0.1 mole of resorcinol in 200 cc. of benzene until evolution of hydrogen chloride ceased. The ester melts at 128° when recrystallized from benzene.

Anal. Calcd. for $C_{13}H_{10}O_4$: C, 67.83; H, 4.35. Found: C, 67.83 and 67.94; H, 4.50 and 4.78.

As mentioned previously under resorcinol di-furylacrylate the full ester (m.p., 112°) is obtained by heating resorcinol with two equivalents of acid chloride. A mixture of the half-ester with the full-ester melted at 95°.

o-Hydroxyphenyl furylacrylate, $C_4H_3OCH=CHCOOC_6H_4OH(o)$, was prepared in accordance with the method described for the preceding ester. The compound melts at 132°.

Anal. Calcd. for $C_{13}H_{10}O_4$: C, 67.83; H, 4.35. Found: C, 67.64 and 67.76; H, 4.72 and 4.74.

It is interesting to note that in an orienting experiment on the preparation of furfuryl oxalate by the Darzen's method (the addition of 0.2 mole of furfuryl alcohol in 50 cc. chloroform to 0.2 mole of the pyridine-oxalyl chloride complex in 200 cc. of dry chloroform) the dark oil obtained subsequent to a thorough washing with water and dil. acetic acid underwent violent and sudden decomposition in an attempted distillation at 4 mm. pressure. There is a possibility that some of the product might have been furfuryl chloride.

⁶ Einhorn, *Buletinul de Chimie*, 17, 110 (1925) [*Zentr.* p. 209 (1916)].

⁷ Zanetti and Beckmann, *J. Am. Chem. Soc.*, 50, 2031 (1928) and Gilman and Dickey, *Iowa State Coll. J. of Sci.*, 5, 193 (1931).

⁸ Provided by a chemical house.

Tetrahydrofurfuryl oxalate, $(C_4H_7OCH_2OCO)_2$. Tetrahydrofurfuryl oxalate was prepared by the method used by Wahl⁹ in the preparation of various esters of oxalic acid. A 8.4 g. (0.0824 mole) portion of tetrahydrofurfuryl alcohol was dissolved in 6 g. of benzene and 3 g. (0.0333 mole) of anhydrous oxalic acid added to the resulting solution. To this mixture was then added 1.79 g. (0.0183 mole) of concentrated sulfuric acid and the mixture stirred at room temperature for 10 hours. The solution was then washed with a small amount of dilute sodium bicarbonate solution, dried over sodium sulfate and distilled. The yield of tetrahydrofurfuryl oxalate boiling at $203^\circ/4$ mm. was 5 g. or 58.4 per cent, based upon oxalic acid.

In a second preparation, 30.6 g. (0.3 mole) of tetrahydrofurfuryl alcohol was added to 9 g. (0.1 mole) of anhydrous oxalic acid and the mixture heated at 120° for 12 hours. The mixture was then distilled to yield 16 g. or 62 per cent, based on oxalic acid, of tetrahydrofurfuryl oxalate boiling at $203^\circ/4$ mm.

This ester is a colorless liquid with little odor; sp.g.₄²⁵ 1.1835; n_D^{25} 1.4650. The saponification equivalent was determined as 127.5, the theoretical value being 129.

Anal. Calcd. for $C_{12}H_{18}O_6$: C, 55.81; H, 6.98. Found: C, 55.79 and 56.05; H, 7.21 and 6.77.

Tetrahydrofurylpropyl oxalate, $(C_4H_7OCH_2CH_2CH_2OCO)_2$. Attempts at preparation of the oxalic ester of tetrahydrofurylpropyl alcohol by the action of anhydrous oxalic acid were not successful. Signs of decomposition were evident and complete decomposition resulted upon attempted distillation at 4 mm. We were, however, successful in securing this compound by the action of oxalyl chloride upon the sodium salt of the alcohol. Thirteen grams (0.1 mole) tetrahydrofurylpropyl alcohol was dissolved in 50 cc. of dry benzene and 2.3 g. (0.1 atom) of sodium added. This mixture was refluxed until no further reaction was evident. The small particle of unused sodium was then removed and 6.35 g. (0.05 mole) of oxalyl chloride was added dropwise with vigorous stirring. The mixture was refluxed for two hours, filtered and distilled to yield 8 g. or 51 per cent of tetrahydrofurylpropyl oxalate boiling at $210^\circ/3$ mm.

This ester is a colorless liquid with little odor; sp.g.₄²⁵ 1.1045; n_D^{25} 1.4675. The saponification equivalent was determined as 157.9, the theoretical value being 157.

Anal. Calcd. for $C_{16}H_{26}O_6$: C, 61.15; H, 8.25. Found: C, 60.63 and 61.00; H, 8.61 and 8.14.

Tetrahydrofurfuryl salicylate, $(C_4H_7OCH_2OCO)C_6H_4OH(o)$. Tetrahydrofurfuryl salicylate was prepared by the action of the acid chloride on tetrahydrofurfuryl alcohol. The salicyloyl chloride was prepared by the method of Adams and Ulrich¹⁰ by the action of oxalyl chloride on salicylic acid. A 13.8 g. (0.1 mole) portion of salicylic acid was dissolved in 50 cc. of dry benzene and 31.8 g. (0.25 mole) of oxalyl chloride added dropwise with stirring. The mixture was refluxed gently for two hours and the excess oxalyl chloride and benzene removed by distillation under reduced pressure. No attempt was made to distill the salicyloyl chloride.

⁹ Wahl, *Bull. soc. chim.*, **35**, 304 (1924); *ibid.*, **37**, 713 (1925).

¹⁰ Adams and Ulrich, *J. Am. Chem. Soc.*, **42**, 604 (1920).

The crude salicyloyl chloride was added to a solution of 10.2 g. (0.1 mole) of tetrahydrofurfuryl alcohol in 100 cc. of benzene and the solution refluxed until evolution of hydrogen chloride ceased. The mixture was then distilled to yield 15 g. or 78 per cent of tetrahydrofurfuryl salicylate boiling at $166^{\circ}/5$ mm. The ester is a colorless liquid with a very pleasant odor; sp.g.^{34}_{20} 1.1831; n^{34}_{D} 1.5217.

Anal. Calcd. for $\text{C}_{12}\text{H}_{14}\text{O}_4$: C, 63.26; H, 5.77. Found: C, 63.25 and 63.57; H, 5.92 and 5.94.

Tetrahydrofurfuryl dichloroacetate, $\text{C}_4\text{H}_7\text{OCH}_2\text{OCOCHCl}_2$. This ester was prepared (after the method described for the preparation of tetrahydrofurfuryl monochloroacetate¹¹) from the alcohol, dichloroacetic acid and hydrogen chloride. It is a stable colorless liquid of practically no odor, but it has a sharp taste. The ester distills at $106\text{--}108^{\circ}/4$ mm.; sp.g.^{20}_{20} 1.3095.

Anal. Calcd. Cl, 31.66. Found: Cl, 31.84 and 31.62.

2-Chloroethyl furoate, $\text{C}_4\text{H}_5\text{OCOOCH}_2\text{CH}_2\text{Cl}$, was prepared by saturating a benzene solution of furoic acid and ethylene chlorohydrin with hydrogen chloride. It distills at $116\text{--}117^{\circ}/9$ mm.; sp.g. 1.2952; has an odor similar to that of ethyl furoate; and has a sharp, pungent taste.

Anal. Calcd: Cl, 20.31. Found: Cl, 19.95 and 20.05.

Tetrahydrofurfuryl β -furylacrylate, $\text{C}_4\text{H}_7\text{OCH}_2\text{OCOCH}=\text{CHC}_4\text{H}_3\text{O}$, was prepared after the method used for tetrahydrofurfuryl acetate. The yield of compound distilling at $163\text{--}167^{\circ}/4$ mm. (sp.g.^{20}_{20} 1.1450) 84.7 per cent.

Anal. Calcd: C, 64.83; H, 6.36. Found: C, 64.95 and 64.90; H, 6.20 and 6.24.

Tetrahydrofurfuryl cinnamate, $\text{C}_4\text{H}_7\text{OCH}_2\text{OCOCH}=\text{CHC}_6\text{H}_5$, prepared like the preceding ester, distilled at $180\text{--}181^{\circ}/4$ mm.; sp.g. 1.120.

Anal. Calcd: C, 72.37; H, 6.94. Found: C, 72.05 and 72.14; H, 6.75 and 6.81.

2-Chloroethyl β -furylacrylate, $\text{C}_4\text{H}_3\text{OCH}=\text{CHCOOCH}_2\text{CH}_2\text{Cl}$. This ester was obtained in 80 per cent yield and distilled at $130\text{--}132^{\circ}/4$ mm.; sp.g.^{20}_{20} 1.2343.

Anal. Calcd: Cl, 17.68. Found: Cl, 17.56 and 17.50.

DISCUSSION OF RESULTS

An examination of Table I reveals that on the basis of flies inactivated in a ten minute period the range of effectiveness of the furan compounds investigated extends from no inactivation with ethyl bromofuryl-dibromopropionate to 67 per cent with *p*-cresyl furoate.

The simpler compounds like Numbers 1 and 2 are relatively ineffective, and yet phenyl furoate (No. 15) is reasonably effective.

In general, those compounds having lateral unsaturation (and, in particular, those with conjugated unsaturation) are effective. Some illustrations are compounds 4, 6, 9, 10, 22 and 24. However, any sweeping

¹¹ Gilman, Hewlett and Dickey, *Iowa State Coll. J. Sci.*, 6, 137 (1932).

generalization concerning the effect of unsaturation is unwarranted in view of the low results with compounds 8, 23, 26, 27 and 28.

Tetrahydrofurfuryl esters (12, 13 and 14) appear of little promise.

There are marked variations between some position isomers. For example, *m*-cresyl furoate is of slight activity and *p*-cresyl furoate is of marked activity. This relative order is maintained elsewhere as in the lesser activity of *m*-cresyl furylacrylate when compared with the isomeric *p*-cresyl furylacrylate.

The introduction of bromine has a general lowering effect and this is particularly noteworthy with 5-bromofurylacrylic acid (No. 10) and 5-bromofuryl-bromoacrylic acid (No. 11). A part of such differences is undoubtedly due to decreased volatility.

In general, the several compounds have comparable ratios of inactivation for the ten and thirty minute intervals, although there are occasional marked exceptions.

The authors are grateful to Professor C. H. Richardson for assistance.

SUMMARY

A preliminary study has been made of the insecticidal action on flies of some furan compounds.

DECARBOXYLATION TEMPERATURES OF SOME FUROIC ACIDS

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INTRODUCTION

The interesting studies by Norris^{1a} on the decarboxylation temperatures of some malonic acids (incidental to a general study on the reactivity of atoms and groups in organic compounds) suggested a related investigation of the decarboxylation of furoic acids. Furoic acids, in general, undergo relatively ready decarboxylation and the smoothness of this reaction with 2-furoic acid commends it as the best present method for the preparation of furan.² It was felt that such a procedure might throw light on (1) the so-called super-aromatic properties of furan³ and (2) on the position of nuclear substituents in some furoic acids of uncertain constitution. The easier thermal decarboxylation of *alpha*-furoic acids and also the more ready replacement of an *alpha*-carboxylic group by mercury² may or may not be related to the greater aromaticity of the *alpha*- as contrasted with the *beta*-position in furan. Although studies by W. E. Catlin on the ionization constants of furoic acid show 2-furoic acid to be more highly ionized than either 3-furoic acid or benzoic acid it is quite doubtful whether sound deductions can be drawn from decarboxylation temperatures and ionization constants^{1b}, even though ionization constants appear to be correlated with aromaticity.

Unfortunately, whatever prospects might have been entertained for applying decarboxylation temperatures to constitution could not be realized with some critical halogen-substituted furoic acids because of secondary decompositions leading to the evolution of halogens and halogen acids. However, it does appear from the present results that the method might be used to correlate thermal decarboxylation in the furan series with substituted malonic acids if a series of 5-alkyl-furoic acids be used. Furthermore, the method or its simple barium hydroxide alternative^{1a} should prove useful in selecting optimal catalytic conditions for the synthesis of furan compounds by decarboxylation.

EXPERIMENTAL PART

The general technique was that used by Norris and Young,^{1a} and the later modifications^{1b} came at a time when most of the work was completed. Two minor experimental changes were introduced: (1) an electric tapping

¹ (a) Norris and Young, *J. Am. Chem. Soc.*, **52**, 5066 (1930). (b) See, also, a related study by Marshall, *Rec. trav. chim.*, **51**, 233 (1932) on the thermal stabilities of some alkylated malonic acids.

² Gilman and Lousinian, *Rec. trav. chim.*, **52**, 156 (1933).

³ Gilman and Towne, *ibid.*, **51**, 1054 (1932).

device was used to facilitate readings of the mercury slug; and (2) in addition to a motor stirrer in the heating chamber, vertical stirring was effected by a vacuum pump arrangement. The results given in Table I and the foot-notes which accompany it are the averages of several determinations for each acid. Because of the instability of some furan compounds and the marked effect of impurities on the temperature of decarboxylation, no claim can be made for quantitative exactness.

TABLE 1. *Decarboxylation temperatures of some furoic acids*

Compound	Melting point	Decarboxylation temperature
2-Furoic Acid	132	158
5-Methyl-2-Furoic Acid	107	122-125(a)
5- <i>tert.</i> -Butyl-2-Furoic Acid	105	125-127
5-Chloro-2-Furoic Acid	175	180-182
5-Bromo-2-Furoic Acid	185	177-179
3,5-Dichloro-2-Furoic Acid	156-157	168-170
3,5-Dibromo-2-Furoic Acid	168-168.5	174(b)
5-Iodo-2-Furoic Acid	193	(c)
5-Nitro-2-Furoic Acid	186	201-203
3-Furoic Acid	122	152
2-Methyl-3-Furoic Acid	101	101-103
2,4-Dimethyl-3-Furoic Acid	114	129(d)
2-Methyl-5-Nitro-3-Furoic Acid	154.5	172
2,4-Dimethyl-5-Nitro-3-Furoic Acid	182	185-186
<i>o</i> -Anisic Acid [<i>o</i> -CH ₃ OC ₆ H ₄ CO ₂ H]	98	213-215
<i>p</i> -Anisic Acid [<i>p</i> -CH ₃ OC ₆ H ₄ CO ₂ H]	106-7	243-245

- (a) The 5-methyl-2-furoic acid gave curves indicating some disturbances at the melting point and the curves did not completely recover to the normal curve before decarboxylation set in. With this in mind a determination was made of that temperature required to give a precipitate when the effluent gases were passed into barium hydroxide. This temperature was 133 or eight to ten degrees higher than that obtained from the curves. Prior to this the related technique of Norris was essentially checked on malonic acid, the widest difference being but two degrees.
- (b) The evolved gases contain some hydrogen bromide.
- (c) Iodine was liberated at about 178°.
- (d) In an orienting experiment inconclusive results were obtained with 2,5-dimethyl-3,4-furandicarboxylic acid which melted at 232° and started to decarboxylate at 80°. The low temperature of decarboxylation is to be verified; however, it is significant that this dibasic acid can be decarboxylated in large quantities with great ease.

DISCUSSION OF RESULTS

The graphs from which the decarboxylation temperatures were taken did not always show a sharp or distinct break in the curve and this accounts for the temperature range in a few cases. This occasional lack of sharpness is not due, we believe, to the method but is due rather largely to the extra thermal sensitivity of some furan compounds.

In general, the decomposition temperatures of malonic acids lie closer to the melting points than is the case with the furoic acids.

The number of alkyl 2-furoic acids reported at this time is altogether

too small to warrant any definite correlations with related malonic acids. However, the two compounds examined indicate a relationship with 2-furoic acid like that observed with malonic acid and its alkyl derivatives. It should be remembered that our butyl-furoic acid is *tertiary* and not *normal*.

The closely related 5-bromo-, 5-chloro- and 5-iodo-2-furoic acids cannot be compared because of secondary decompositions. One conclusion can, however, be drawn and that is the general or over-all relative stabilities of these compounds: The iodo being least stable and the chloro most stable. For this reason no decarboxylation temperature is reported with the iodo compound. The decarboxylation temperature of the bromo compound is of no great significance since it is compounded of several factors only one of which is decarboxylation, the others involving expulsion of the halogen and secondary reactions of such halogen and halogen acid on the resulting compounds like bromofuran.

The difference between the decarboxylation temperature and melting point of 2-furoic acid ($158 - 132 = 26$) is slightly less than that with 3-furoic acid ($152 - 132 = 20$). Somewhat related to this is the fact that preferential decarboxylation of an *alpha-beta*-furandicarboxylic acid (2,4-furandicarboxylic acid) gives quite satisfactory yields of 3-furoic acid. The occasional anomalous "breaks" with 3-furoic acid are being investigated further.

The *o*- and *p*-methoxybenzoic acids were investigated in connection with some correlations on the super-aromatic properties of some benzene and furan types.

The authors are grateful to George F. Wright for assistance.

SUMMARY

The Norris graphical method for determining the temperatures of decarboxylation of malonic acids has been applied to a number of furoic acids in connection with a study of the relative reactivity of elements and groups attached to the furan nucleus.

THE TAXONOMIC POSITION OF AN AROMA-PRODUCING MOLD

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A taxonomic study of a mold of the Moniliaceae, isolated by Dr. Max Levine from corn mash, is presented. The mold was characterized by a peculiar fruity aroma which it produced when grown on wort agar.

In the literature, descriptions of two molds were found which corresponded sufficiently with this species to warrant closer comparison. The first was that of Lindner (1) which he called his "wine-bucket" mold and named *Sachsia suaveolens*. Later Lindau (2) placed this organism in the genus *Oospora* and called it *Oospora suaveolens*. A short time after this Krzemecki (3) isolated a mold characterized by a similar fruity aroma from a sample of water. Lindner compared the latter with his and pointed out that they were not the same. Krzemecki then named his mold *Oidium suaveolens*. Since these genera are members of the Moniliaceae and there is more or less confusion concerning them, a study of the mold was made and its morphology and physiology compared with the descriptions given by Lindner and Krzemecki, with a view to properly allocating the organism.

MORPHOLOGY

The mycelium is typically mold-like. The hyphae are hyaline in color and have a diameter of from 4 to 5 microns. In the growing hyphae, branches appear laterally and posteriorly to the septa soon after they are formed. Branching at the tips of the hyphae does not occur. Budding was not observed. In the isolated colony the filaments appear to radiate from the center. (Plate I, A) In the developing isolated colony the hyphae become fasciculated and branching occurs only on the outside of the bundles. (Plate I, D) These outside branches also become fasciculated. This characteristic of fasciculation results in pseudo-coremia formation on the surface growth. (Plate I, B and C) When the tips of the hyphae come into contact with the glass surface around the culture they appear to flatten out in irregular finger-like projections and form appressoria. From the irregular projections of these appressoria, filaments grow back into the medium. (Plate I, D and Plate II).

Conidia are formed by the segmentation of the hyphae, both in the pseudo-coremia and in the medium. The conidia are cylindrical in shape with rounded corners, and when observed in cultures on solid media, appear to consist of irregular zig-zag chains of cells. Conidia formed in young cultures present a granular appearance and show oil drops in the ends of the cells. Photomicrographs (magnification of 500 diameters) were made of the conidia and their size measured. A variation in length of from 10 to 30 microns was found, the average being 21 microns. The diameter was between 4 to 5 microns. (Plate I, E and Plate II, D).

¹ The writer wishes to express his appreciation to Dr. J. C. Gilman and Dr. Max Levine for their interest, cooperation and helpful suggestions in this work.

CULTURAL CHARACTERS

The surface growth is much the same on the various media. The chief difference is in the vigor of growth and a correspondingly greater or less degree of spreading and pseudo-coremia formation. The organism grew well at a temperature of 23° to 26° C., poorly at 37° C., and gave no growth at 40° C. The mold was grown on the following media:

Potato Dextrose Agar (Difco) Slants: Vigorous growth with strong aroma production. The growth spreads and abundant pseudo-coremia are formed.

Wort Agar (Difco) Slants: Vigorous spreading growth, producing pseudo-coremia and strong aroma.

Potato Slants: Vigorous spreading growth and pseudo-coremia production; slight aroma.

Bean Agar (Difco) Slants: Moderate growth with slight spreading. Hyphae extend deep into the medium. Pseudo-coremia, if formed, are small; slight aroma.

Whey Agar (Difco) Slants: Very poor growth.

Nutrient Agar (Difco, Standard Methods Formula) Slants: Very poor growth.

Nutrient Gelatin: The organism grew moderately well; medium not liquefied.

Starch Agar Plates: Good growth. The starch was not hydrolyzed.

Lead Acetate Agar (Difco) Stab: Moderate growth on the surface and along the stab. Hydrogen sulfide was not formed.

Malt Extract Broth (Difco): Vigorous growth and strong aroma.

Peptone Water and Nutrient Broth: Only a fair growth was obtained. An occasional slight trace of aroma was noted.

Nitrate Broth: Moderate growth with no aroma. The test for nitrites was negative.

Litmus Milk: Moderate growth and aroma production. There was no change in reaction.

Growth was scanty or moderate and neither acid nor gas was produced in broth containing lactose, mannitol, trehalose, melizitose, pectin, rhamnose, salicin, sorbitol, xylan, xylose, gum arabic, starch or cellulose. Vigorous growth with acid production and strong aroma was obtained in dextrose, sucrose and glycerol broth. The initial reaction was pH 7.0.

GROWTH OF A CONIDIUM

A moist chamber was employed in the study of the developing conidium. The medium used was dextrose tartaric acid agar. The culture was prepared by touching the tip of an inoculating needle to a well shaken malt extract broth culture of the mold and then touching the center of a sterile cover slip with the needle. A loop of the melted agar was then placed on the inoculated area and the cover slip mounted on the moist chamber. The time of making the preparation was recorded and a search made for a conidium. Figure 1 shows the photomicrographic record of the growth for thirteen hours.

The following points of interest were noted from this photographic study. Growth appeared at one end of the conidium in 55 minutes, the resulting filament showing initiation of appressorium formation in six and one-half hours. Growth from the opposite end of the same conidium de-

veloped in five and one-half hours. In this latter filament a different type of cross wall from those previously observed, namely, a very heavy septum, was formed at 10 and 11 hours as may be seen in figure 1. This filament then separated at this point and became segmented, new growths developing from the ends of the separated cells as is shown in the photograph made at thirteen hours.

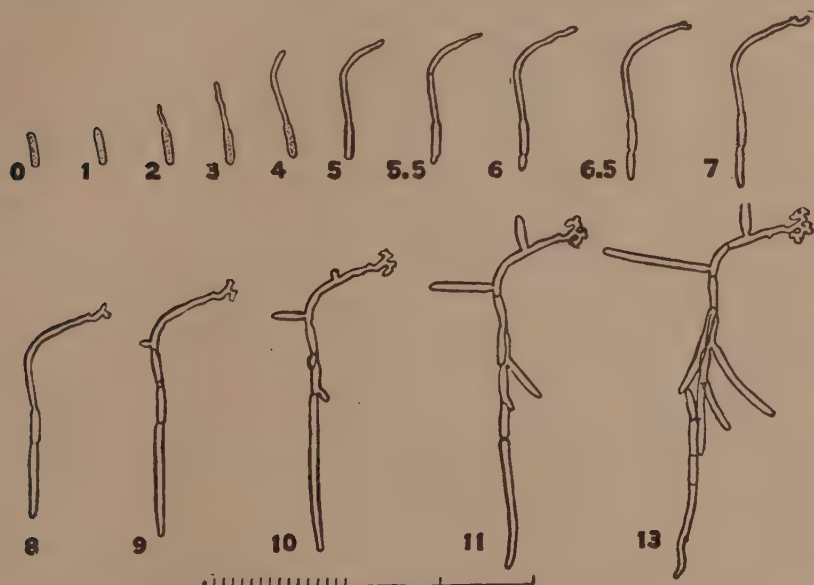


Fig. 1. Development of a conidium. (Numbers indicate time elapsed in hours. Scale: — 1 unit = 5μ).

Measurement of the hourly growth of each branch as it developed was made. In table 1 the hourly increase in length of each branch is tabulated. The average increase in length of all filaments was 17.2 microns per hour.

TABLE 1. *Elongation of hyphae in microns per hour*

Age of branch in hours	Order of branching					
	1	2	3	4	5	6
1	14 μ	13 μ	12 μ	17 μ	22 μ	15 μ
2	11 μ	19 μ	22 μ	24 μ		
3	15 μ	20 μ	16 μ			
4	17 μ	21 μ				

TAXONOMY

Taxonomically, this organism might be considered as a member of one or other of the four genera of the Moniliaceae: *Oidium*, *Sachsia*, *Oospora*

or *Cylindrium*. The characterizations that have been given these genera in the past has been so varied that today there results a great deal of confusion. An analysis of the characters of this mold and that of Krzemecki, called *Oidium suaveolens*, shows them to be the same. Table 2 presents a comparison of the characters of the molds described by Krzemecki, Lindner and the one now presented. The same characters of *Oospora lactis* are included to show possible relationships. In order to make clear the reason for changing the taxonomic position of this mold a brief outline of the modern concept of these genera is given.

TABLE 2. *Comparative characters of mold forms considered*

Character	New culture	Krzemecki	Lindner	Oospora lactis
Mycelium				
Branching	Lateral	Lateral	Apical Lateral	Dichotomous
Budding	Absent	Absent	Present	Présent
Appresoria	Present	Present	Absent	Absent
Fruiting Hyphae				
Branching	Absent	Absent	Present	Present
Pseudo-coremia	Present	Present	Absent	Absent
Conidia	Cylindrical	Cylindrical	Globose	Globose

Today the genus *Oidium* is considered as limited to the plant parasitic species which are known to be the conidial stages of Erysiphaceae. Since this organism is clearly a saprophyte the name proposed by Krzemecki cannot now be used.

Sachsia and *Oospora* are closely related to *Oidium* and are now regarded as containing saprophytes. Lindau considered that the genus *Sachsia* should not be separated from *Oospora* for the reason that the characteristic of budding which was the supposedly distinguishing character for *Sachsia* occurred in both genera. For this reason he placed Lindner's aromatic mold, *Sachsia suaveolens*, in the genus *Oospora*.

In 1923 Berkhout (4) attempted a reorganization of this family. In this publication she placed Krzemecki's mold in the genus *Oospora*. Lindner had compared Krzemecki's culture with his own and found that it was not *Oospora suaveolens* (Lindner) Lindau, and so Berkhout was forced to give it a new name, namely, *Oospora fragrans*, Berkhout. She apparently overlooked the fact that the fourth genus *Cylindrium* which differs from *Oospora* by containing those forms with cylindrical rather than globose conidia might better fit this organism. It is in this group that our mold should be placed, as is clear from the shape of the conidia (Pl. I. fig. E; Pl. II. fig. D.). It is therefore proposed that a return be made to Krzemecki's original specific name with the new combination, *Cylindrium suaveolens*, n. comb.

The organism may be characterized thus:—Colony gray, membranous; surface fasciculated with pseudo-coremia formation, hyphae hyaline, very short and little differentiated from the conidia; mycelial threads radiate from center and present lateral branching; appresoria present; conidia

cylindrical with slightly rounded corners, varying in size from 10-30 μ (Av. 21 μ) x 4-5 μ .

SUMMARY

Characters of an aroma-producing mold, similar to *Oidium suaveolens* Krzemecki, have been presented. Discrepancies in the taxonomy have been pointed out and a new combination, *Cylindrium suaveolens* proposed.

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Plate I

Fig. A. Subsurface colony showing radiation hyphae.

Fig. B. Pseudo-coremia.

Fig. C. Pseudo-coremia as seen from the side.

Fig. D. Developing colony showing fasciculation.

Fig. E. Conidia.

Plate I

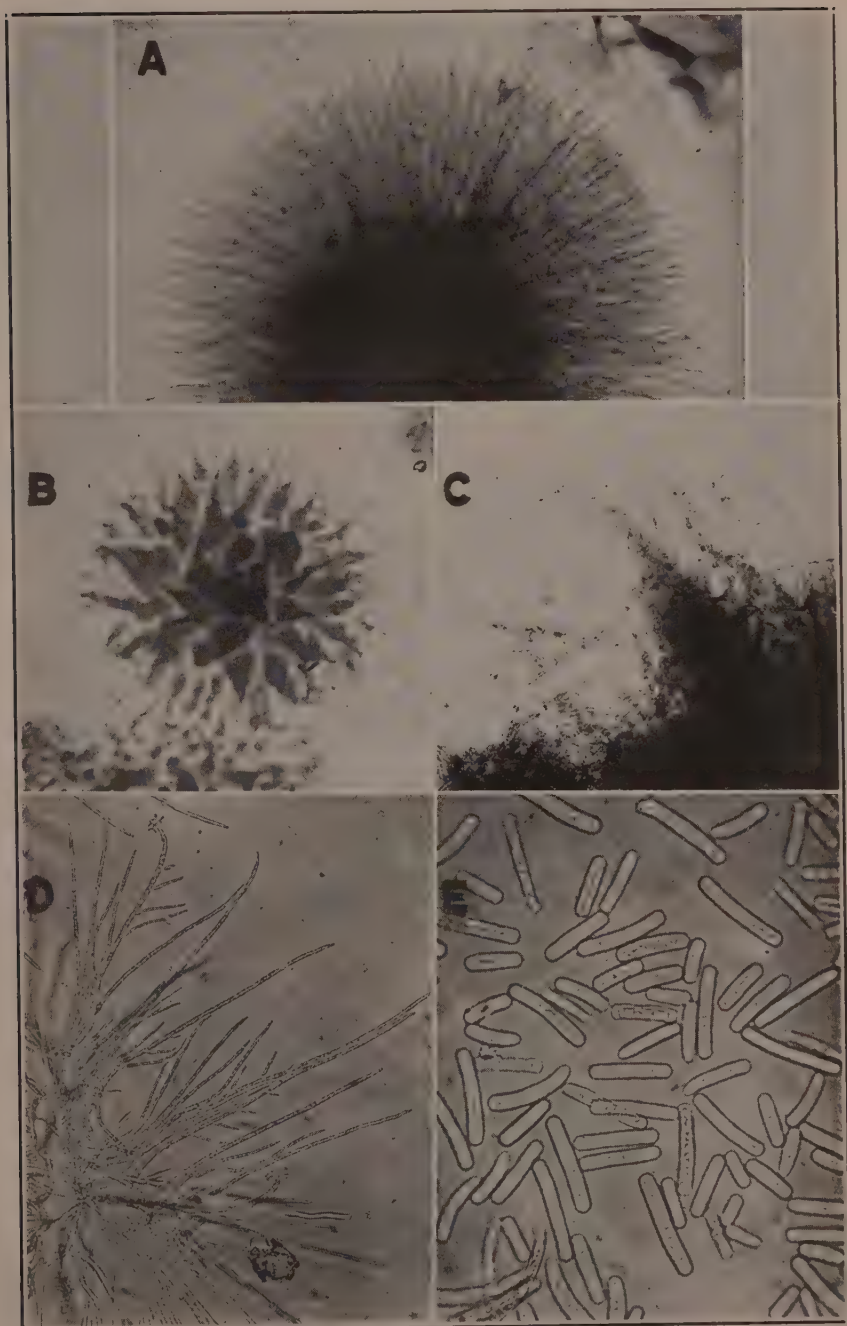
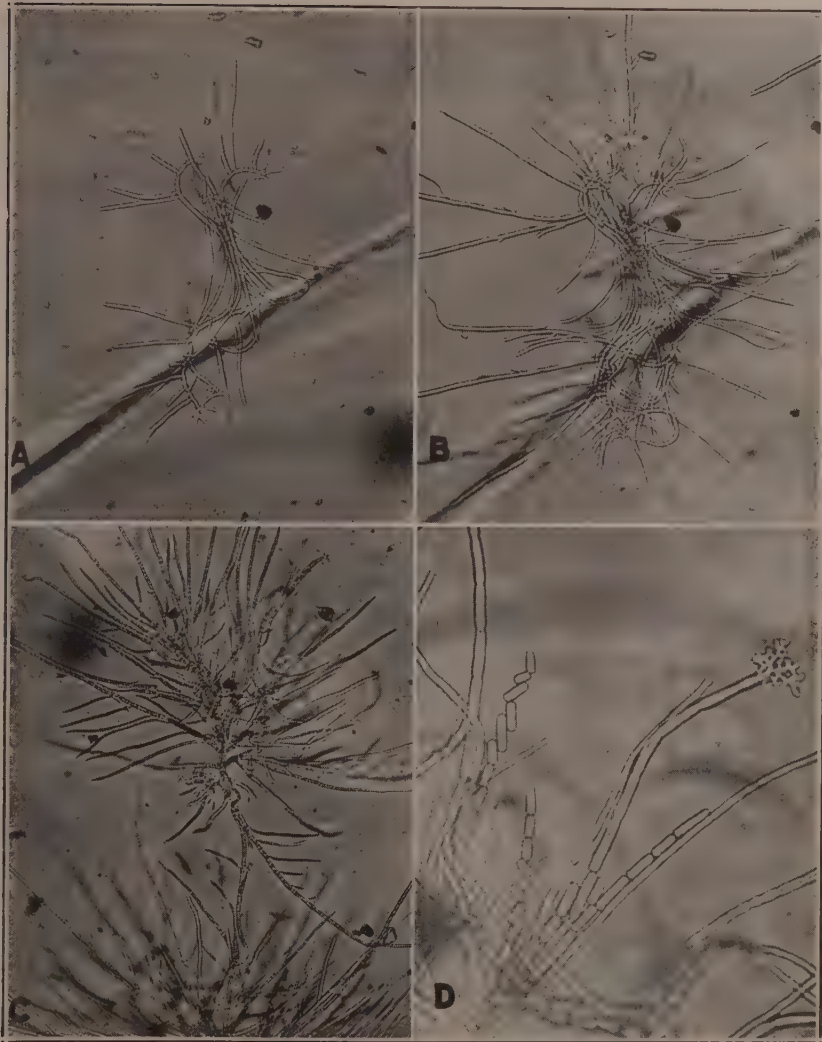


Plate II

- Fig. A. Young colony showing appresoria.
- Fig. B. Same colony as (A) 12 hours later showing filaments growing from appresoria.
- Fig. C. Lateral branching and appresoria with developing filaments.
- Fig. D. Appresoria and conidia formation shown in more detail. Note zig-zag arrangement of cells.

Plate II



DETERMINATION OF THE FURFURAL YIELDING CONSTITUENTS OF PLANT MATERIALS¹

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In studies on the fermentation of agricultural wastes the problem has been met of determining the relative quantities of furfural yielding constituents (pentoses and pentosans) before and after completion of the fermentation. A survey of the methods available for the determination of these materials shows that the method most widely used is that based upon the work of Krober and Rimbach (14), which is the standard method recommended by the Association of Official Agricultural Chemists (1). As has been pointed out by a number of investigators, the method has several disadvantages, chief among them for the present problem being the time required for completion of the analysis.

The conversion of pentose materials to furfural by Krober's method is based on an empirical method of distillation from 12 per cent HCl. The sample is placed in a 250 ml. flask with 100 ml. of 12 percent HCl and distilled at such a rate that 30 ml. of distillate are collected in ten minutes. The acid is replaced by adding another 30 ml. of 12 per cent HCl. through a dropping funnel and the distillation continued at the same rate with further additions of acid at ten-minute intervals until 360 ml. of distillate have been collected. A quantity of phloroglucinol solution in 12 per cent HCl, containing about twice the required amount of the precipitant, is added to the distillate, the total volume made up to 400 ml. with 12 per cent HCl and allowed to stand for fourteen hours. The precipitate is then collected on a prepared Gooch crucible, washed with 150 ml. of distilled water (in such a manner that the water is never completely removed from the crucible until the last), dried for four hours at 100°C. and weighed in a weighing bottle. The weight of phloroglucid obtained is converted to pentose or pentosan by reference to Krober's tables (1, 13).

In the original work of Krober and Rimbach (14) it was shown that when furfural solutions are precipitated by phloroglucinol as described, 0.0052 gm. of phloroglucid remains in solution and is dissolved by the 150 ml. of wash water. The data upon which the conversion tables were built, were then obtained by distillation of samples of pure xylose and arabinose in the prescribed manner, results being obtained as shown in table 1.

TABLE 1. *Ratio of xylose to phloroglucid obtained by Krober and Rimbach*

Xylose	Phloroglucid	Ratio phloroglucid xylose
0.050	0.04925	1:0.985
0.100	0.1040	1:1.040
0.200	0.2137	1:1.068
0.250	0.26875	1:1.075

The weight of phloroglucid thus obtained, corrected for the amount remaining in solution, resulted in a more constant ratio, as shown in table 2.

TABLE 2. *Ratio of xylose to corrected weight of phloroglucid*

Xylose	Phloroglucid	Phloroglucid + 0.0052	Ratio
0.050	0.04925	0.05445	1:0.9183
0.100	0.1040	0.1092	1:0.9158
0.200	0.2137	0.2189	1:0.9137
0.250	0.2687	0.27395	1:0.9320

From these ratios and similar developed ratios for arabinose, the following formulas used for construction of the conversion tables were derived:

$$\text{Xylose} = (a + 0.0052) \ 0.9183 \text{ to } 0.9320$$

$$\text{Arabinose} = (a + 0.0052) \ 1.1086 \text{ to } 1.0941$$

where "a" equals the weight of phloroglucid obtained.

It is obvious that the tables depict only direct relationships between weight of pentose and phloroglucid obtained by rigid adherence to the prescribed technique, and although we frequently find reports in the literature in which the results obtained by other methods of distillation have been interpreted by means of Krober's conversion tables, there can be no justification for the practice.

Conversion of the pentose materials to furfural by steam distillation until exhaustion of furfural as recommended and used by a number of workers has some evident advantages over the above method, but has hardly been sufficiently standardized for practical use. Several investigators, Jolles (4), Ling and Nanji (6), and Pervier and Gortner (8), using various methods for determining furfural, reported 100 per cent conversion of pure xylose and arabinose by steam distillation from dilute HCl. Since other investigators have not succeeded in obtaining 100 per cent conversion of both xylose and arabinose by any method of acid distillation it is apparent that such theoretical conversions cannot be consistently obtained. The use of a method involving steam distillation, then, is dependent upon the development of proper conversion factors as in the standard method.

Various other modifications of the distillation have been proposed while a great variety of methods and reagents have been used for estimating the furfural content of the distillate. It is not, however, within the scope of this paper to give a complete review of the subject. A rather complete bibliography of reports dealing with the determination, up to that time, is given by Pervier and Gortner (8). These workers, applying the steam distillation method of Jolles (4) to the conversion of xylose and arabinose to furfural and determining the furfural by an electrometric bromate titration reported 100 per cent recovery of these two pentoses. At the same time they demonstrated that when distillation is carried out by the standard method, the acid concentration in the distillation flask rises rapidly and after 60 ml. of distillate have been collected remains between 18 and 20 per cent HCl. They could explain their high yields, then, only on the basis of rapid removal of the furfural by steam with consequent prevention of its destruction by longer contact with the strong acid.

It remained, however, for Kline and Aerce (5) to prove that practically 100 per cent recovery of furfural from solution could be obtained by both the standard and steam distillation methods and that the degree of conversion of xylose by both methods is practically the same. On 0.0697 gm. samples of xylose they obtained 88 per cent recovery by each of the two methods.

These results suggested to us the possible advantages offered by using a constant boiling or 20 per cent HCl mixture in a rapid distillation method for converting pentose materials into furfural.

The furfural was precipitated with 2,4-dinitrophenylhydrazine and weighed as the corresponding hydrazone.

The use of 2,4-dinitrophenylhydrazine has been found to be very satisfactory as a precipitant for a number of carbonyl compounds and it seemed desirable to determine its applicability to the determination of furfural under these conditions. The solution of 2,4-dinitrophenylhydrazine was prepared by saturating cold dilute (2 normal) HCl with the reagent and filtering immediately before using. The solution prepared in this manner contains about 4.2 mg. of 2,4-dinitrophenylhydrazine per ml. and should be freshly prepared since the reagent undergoes decomposition on standing. The furfural solutions were prepared from the constant boiling fraction obtained by two distillations, under reduced pressure, of C. P. furfural (Pfanstiehl).

Precipitation of the furfural was carried out by adding an excess of the reagent to the furfural solution in 20 per cent HCl. The precipitate was allowed to stand three hours or longer at room temperature, collected on a Gooch crucible, washed with dilute (2 normal) HCl, then with water and weighed after drying at 100°C. for one hour.

Comparisons were made with the bromide-bromate titration of Powell and Whittaker (9) which has been recommended by Kline and Aerce (5). The determinations by this method were carried out in 12 per cent HCl as described by Powell and Whittaker. The results, table 3, show close agreement with those obtained by precipitation with 2,4-dinitrophenylhydrazine.

TABLE 3. *Comparison of furfural determinations by the method of Powell and Whittaker with precipitation as the 2,4-dinitrophenylhydrazone*

Weight of furfural	Weight of furfural found	
	Precipitation	Titration
.0039	.0034	.0042
.0077	.0076	.0079
.0194	.0191	.0193

Simon (10) has used 2,4-dinitrophenylhydrazine to determine the furfural in distillates obtained by distilling pentoses from 12 per cent HCl according to Krober's method. He gives analytical data showing the composition of the precipitate to agree very closely with the theoretical formula

$C_{11}H_8N_4O_5$, so that the factor $\frac{\text{furfural}}{2,4\text{-dinitrophenylhydrazone}}$ equals .3478.

His results show that precipitation is complete in one hour if held in a freezing mixture. We have confirmed the more rapid precipitation at lower temperatures and have found it to be complete in two hours when held at 5 to 10°C. in the ice-box.

Preliminary experiments on the distillation of pure xylose from 20 per cent HCl gave varying yields when a given volume of distillate was collected at varying rates of distillation. Further experiments, however, gave constant results when distillation was carried out at a fixed rate from a solution held at a constant volume of 100 ml. by adding 20 per cent HCl from a dropping funnel. The maximum yield of furfural from xylose was obtained when 200 ml. of distillate were collected in 60 minutes. Distillations were made from a constant volume of 100 ml. and the results of four experiments are shown in table 4.

TABLE 4. *Weights of 2, 4-dinitro phenylhydrazone of furfural obtained by distilling xylose in 20 per cent HCl and collecting 200 ml. of distillate in 60 minutes*

Experiment	1	2	3	4
Weight xylose gms.	0.1016	0.1000	0.3013	0.0291
Distillation time for 200 ml. dist. min.	58	60	60	60
Weight of precipitate gms.	0.1608	0.1576	0.4664	0.0470
Furfural equivalent gms.	0.0559	0.0548	0.1622	0.0163
Theoretical furfural gms.	0.0650	0.064	0.1928	0.0186
Percentage recovery	86.0	85.6	84.1	87.6
Distillation time for additional 100 ml. of dist. min.	30	30	30	30
Weight precipitate	0.0000	0.0000	0.0002	0.0000

The method as proposed, then consists of the following steps:

1. Place the sample in a 250 ml. Erlenmeyer flask with 100 ml. of 20 per cent HCl and distill at such a rate that 195 to 200 ml. of distillate are collected in one hour. Keep the volume constant in the distilling flask by adding acid from a dropping funnel. Allow the distillate to pass through a small filter paper and collect in a graduated cylinder in order to aid in adjusting the rate of distillation.

2. To the total distillate or an aliquot add an excess of 2,4-dinitro-phenylhydrazine solution (prepared by saturating cold, 2 normal HCl with the reagent and filtering immediately before using). Allow the precipitate to stand three hours or longer at room temperature or two hours in the refrigerator.

3. Collect the precipitate on a Gooch crucible, dry one hour at 100°C. and weigh.

We have found that the rate of distillation can be best controlled by the use of an electric heater with adjustable rheostat.

A number of distillations on samples of pure xylose and arabinose gave the results shown in table 5. In each case the results are the average of four or more closely agreeing determinations.

These results show a small but regular increase in the percentage of recovery as the concentration of pentose decreases.

In contrast to this, results as reported in Krober's tables show an increasing yield in the opposite direction i.e., increasing percentage of recovery with increasing concentration of pentose. The values in table 6 are taken from Krober's tables.

The same contrast is to be found in results reported by Kline and Acree (5), in which furfural aliquots from the same distillations were determined by precipitation with thiobarbituric acid and by the bromine titration, the percentage recovery by the second method being in inverse order of that by the first. Their results are shown in table 7.

TABLE 5. *Weights of 2, 4-dinitro phenylhydrazine and percentage recovery of xylose and arabinose by distillation from 20 per cent HCl*

	Theoretical furfural mg.	Wt. of precipitate mg.	Furfural equivalent mg.	Percentage recovery
Arabinose mg.				
30	19.2	40.3	14.0	73.0
50	32.0	66.7	23.20	72.5
100	64.0	131.2	45.5	71.2
200	128.0	258.5	89.7	71.4
300	192.0	375.2	130.5	68.0
Xylose mg.				
30	19.2	48.3	16.8	87.5
50	32.0	80.5	28.05	87.0
100	64.0	159.7	55.5	86.7
200	128.0	312.1	108.8	84.8
300	192.0	458.0	159.3	82.9

TABLE 6. *Weights of phloroglucid and percentage of recovery of xylose by Krober*

Wt. xylose	Wt. phloroglucid	Furfural equivalent	Theoretical furfural	Percentage recovery
.0324	.030	.0182	.02075	87.7
.0681	.069	.0385	.0436	88.2
.1046	.109	.0593	.0669	88.6
.2784	.300	.1581	.178	88.8

Since it has been shown by Kline and Acree that the bromine titration is accurate for small amounts of furfural and we have found that results obtained by precipitation with 2,4-dinitrophenylhydrazine agree closely with those obtained by the bromine titration, it is evident that the decreased yields of furfural in the distillation of small samples when determined by precipitation with phloroglucinol or thiobarbituric acid must be due to the

incomplete precipitation by these reagents, particularly in the more dilute solutions.

TABLE 7. *Percentage recovery of xylose when furfural determined by precipitation with thiobarbituric acid is compared with the bromine titration as reported by Kline and Acree*

Wt. xylose	Xylose found—Percentage recovery	
	Precipitation by thiobarbituric acid	Bromine titration
.0139	29.10	93.53
.0697	88.02	91.93
.1393	83.05	85.06
.2786	83.29	81.52

From the data obtained on the distillation of xylose and arabinose (table 5) from 20 per cent HCl, the following equations for converting the weights of precipitate to the respective pentoses were derived.

$$(1) \text{ Weight of arabinose} = \frac{x}{0.73 - [(x - 40.3) (0.00015)]} \cdot 0.5433$$

$$(2) \text{ Weight of xylose} = \frac{x}{0.87 - [(x - 48.3) .000112]} \cdot .5433$$

where x = weight of precipitate.

Considering xylan and araban as the anhydrides of the respective pentoses, weights of the pentoses can be converted to xylan or araban by multiplying by the factor

$$\frac{C_5H_8O_4}{C_5H_{10}O_5} = \frac{132}{150} \text{ or, xylan} = \text{xylose (0.88)}$$

araban = arabinose (0.88).

In analysis of plant materials where the type of sugar is not known it is common practice to report the average of values calculated as arabinose and xylose as pentoses and the average of araban and xylan as pentosan.

Determinations were carried out upon samples of two xylan preparations and upon samples of beet pulp and ground artichoke tubers by Krober's method and by distillation from 20 per cent HCl. The results obtained are shown in table 8.

TABLE 8. *Comparison of pentosan determinations by Krober's method with the proposed method*

Material	Krober's method	Authors' method
Xylan A	81.76 per cent xylan	82.4 per cent xylan
Xylan B	75.67 " "	75.55 " "
Beet pulp	19.04 " pentosan	19.07 " pentosan
Artichoke tubers	4.15 " "	4.36 " "

DISCUSSION

No attempt at a final solution of the problem of pentose determinations in complex mixtures is claimed by the present presentation, and the method is empirical as any method must be which cannot rest upon 100 per cent conversion. Kline and Acree and other workers have suggested the desirability of attacking the problem from the point of obtaining theoretical conversion and attempting to determine the conditions under which such results might be attained. Hurd and Isenhour, (3), however, studying pentose conversion by HCl and H_2SO_4 found that the change of xylose to furfural followed the unimolecular law, and that the rate of conversion could be represented by straight line functions in keeping with the unimolecular equation. As the time increased, though, the furfural yields showed a tendency to decrease and the straight line functions became curved, which would suggest that 100 per cent conversion by ordinary acid distillation will be impossible to obtain.

It is obvious that steam distillation from dilute acid until exhaustion of furfural, would have certain advantages over the shorter method as given here. We believe, however, that any such advantages are more than off-set by the much greater dilution of the furfural solution resulting from steam distillation and the consequent inaccuracies resulting in its determination.

In addition, we are still confronted with the inaccuracies involved in the production of furfural and methyl furfural from other constituents of plant materials by such distillations. Rimbach (12) found that sucrose gave 1.15 per cent calculated as pentosan, potato starch 0.84 per cent and Swedish filter paper 1.45 per cent. Davis and Sawyer (2) corroborated these results, but suggested that the error would be significant only when hexoses were present in considerably greater quantities than the pentoses. They showed that when 0.01 gm. of pure arabinose was mixed with 25 times its weight of sucrose (0.25 gm.), the result was 20 per cent too high. As a consequence of these results, Spoehr (11) and Davis and Sawyer, recommended removal of the hexoses by fermentation and determination of the pentoses remaining after removal of the fermentation products for exact results. However, due to the obvious difficulties involved, the method has not come into general use.

In view of these difficulties, Noll and Belz (7) have suggested that the results obtained by following a specific procedure be reported as the furfural number of the material under consideration. With the above difficulties in mind, though, it makes little difference whether results are reported as pentoses or pentosans or as a furfural number and in any case, interpretation of analytical data must take into consideration possible sources of furfural or related compounds which may influence the determination and give quantities of furfural in excess of that actually obtained from the pentose constituents.

CONCLUSIONS

A method is given for the determination of furfural-yielding constituents of plant materials based on rapid distillation from 20 per cent HCl followed by determination of the resulting furfural solution by precipitation with 2,4-dinitrophenylhydrazine.

Furfural determinations by precipitation have been compared with

those obtained by the bromide-bromate titration of Powell and Whittaker and shown to give the same results.

Comparisons of the results obtained in determinations upon xylan preparations and plant materials by this method and by Krober's method are given and show close agreement.

Advantages claimed for the method as developed, over Krober's method are:

1. The distillation requires much less attention and the time of distillation is shortened from two hours to one hour.
2. Precipitation is complete and no factor is needed to correct for the solubility of the hydrazone.
3. Precipitation with 2,4-dinitrophenylhydrazine is complete in two to three hours, while fourteen hours are required for precipitation with phloroglucinol.
4. The precipitate is readily dried at 100°C. in one hour, while drying of the phloroglucinol requires four hours.
5. The precipitate is not hygroscopic and does not require use of weighing bottle as does the phloroglucinol.
6. The cost of 2,4-dinitrophenylhydrazine is negligible as compared with phloroglucinol.

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MIGRATION OF BACTERIA THROUGH BUTTER¹

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The migration of both motile and non-motile bacteria through liquid dairy products, such as milk, is evident from the usual laboratory cultures. Although the oxygen requirements of some species largely limit their growth to a certain portion of a culture, many others quickly spread through all of the medium available; for example, in milk at a favorable temperature, *Streptococcus lactis* is soon widely distributed regardless of the portion of the milk that was inoculated.

From the standpoint of the growth of microorganisms in it, butter presents conditions that are distinctly different from those in milk. The quantitative relationships of the water and fat in the two products are very different and during the preparation of butter the physical condition of the fat is greatly changed. Regardless of which one of the more plausible theories of the constitution of butter is accepted, the rapid distribution of bacteria through it would not be expected.

In the studies carried out at the Iowa Agricultural Experiment Station on the effect of various bacteria on the flavor and aroma of butter when the organisms are added to the cream from which the butter is churned, the usual procedure has been to remove portions of the butter, for organoleptic tests, with a clean but unsterilized spatula; the assumption has been that if only a few bacteria are added to the butter they will have no effect on the subsequent changes in the product. This procedure seemed to be satisfactory until, in one trial, an organism that was unexpected was found in a lot of experimental butter when it was examined bacteriologically. The previous treatment of the butter was such that the contaminating organism could have been carried to it from another lot by a common spatula. A second examination of the butter confirmed the presence of the organism since it was found in larger numbers than in the first examination. The results led to a study of the migration of bacteria through butter and the data obtained are reported herein.

METHODS

The butter used was regularly unsalted because of the restraining action of salt on the bacteria usually present in butter. Some of the butter was secured from commercial churnings but most of it was churned in the laboratory since, with such butter, contamination can be controlled more completely than under commercial conditions, and small lots of butter can be inoculated by adding an organism to the cream. For the butter churned in the laboratory the cream was either pasteurized or sterilized in a large

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flask and then poured into sterile, quart, glass jars; the churning was carried out in a small experimental churn in which the jars of cream are turned end over end. The butter was washed with sterile water and worked in a sterile dish with a sterile paddle. In some instances poorly worked and well worked butter were compared, the poorly worked sample being taken when the working of a lot of butter was about half completed and the well worked sample when the working was finished.

When a small lot of inoculated butter was prepared by adding an organism to the cream, butter color or Nile-blue sulphate was commonly added to the cream so that there would be a conspicuous difference in color between the butter originally containing the organism and that which did not. The amounts of Nile-blue sulphate used were so small that bacterial development did not appear to be inhibited.

Most of the trials were carried out at approximately 21°C. because of the very rapid growth at this temperature of many of the organisms commonly present in dairy products, but in some instances about 5°C. was used in order to simulate commercial holding conditions.

The presence of the inoculated bacteria at various points in the butter was sometimes determined by culturing on agar slopes, and sometimes by adding a small amount of the butter to a tube of agar that had been melted and cooled to about 40°C. and then pouring the agar into a petri dish. With the latter procedure a general idea of the numbers of organisms in the butter could be secured more satisfactorily than with the former. In the trials with *Streptococcus lactis*, cultures were made also in litmus milk. When butter from a given point was taken for a culture, both surface and subsurface material was included so that the bacteria would be secured whether they were migrating along the surface or through the butter.

In each trial, cultures were made at various times over an extended period so that if migration occurred it would be detected, regardless of the time required.

The bacteria used were of various types and were selected largely on the basis of the ease with which they could be identified when recovered from butter. Most of the cultures were isolated from cream or butter although two (*Serratia marcescens* and *Pseudomonas graveolens*) were secured from culture collections. Except for *Streptococcus lactis* and one of the fluorescent types, all of the organisms were motile and a number of them were lipolytic. A few of the organisms used were not definitely identified: the identified organisms are *Achromobacter lipolyticum*; *Pseudomonas fluorescens*; *Pseudomonas fragi*; *Pseudomonas graveolens*; *Pseudomonas schuylkilliensis*; *Pseudomonas synxantha*; *Serratia marcescens*; *Streptococcus lactis*.

EXPERIMENTAL

The migration studies can be divided conveniently into two parts, (a) the trials in which the butter was inoculated directly from an agar or milk culture, and (b) the trials in which a small portion of the butter was inoculated by adding the organism to the cream from which it was churned.

(a) TRIALS IN WHICH THE BUTTER WAS INOCULATED DIRECTLY FROM AN AGAR OR MILK CULTURE

The trials involving the direct inoculation of butter from an agar or milk culture were carried out in various types of containers, especially

petri dishes and glass cylinders. A small amount of material from an agar or milk culture was transferred to the butter with a needle or loop and mixed into the butter (with the needle or loop) over a small area to a depth of about 0.25 inch; commonly the area involved was definitely indicated by a shallow mark on the surface of the butter. Cultures taken from an area of inoculation at various times usually showed many organisms of the type inoculated, although occasionally with a long holding period the number was comparatively small. The studies in progress at the Iowa Agricultural Experiment Station suggest that in butter which becomes rancid through the action of bacteria there may be a rather rapid decrease in the bacterial content.

Petri dish trials. In the petri dish trials the butter was regularly packed into the dishes with enough space between it and the cover of the dish to be certain the two would not touch. The butter was inoculated near the center of the surface.

In most of the trials the cultures taken outside the inoculated area indicated that there was no movement of the bacteria from this area. Occasionally a very few organisms of the added species were secured from a point near the inoculation, but commonly cultures taken within 0.25 inch of the inoculated area, as well as at greater distances, failed to show any evidence of migration. In the few instances in which migration occurred, poorly worked butter was involved more frequently than well worked butter.

A number of the trials were carried out with the butter held at about 5°C.; none of them gave any evidence that the added organisms were outside the area of inoculation.

The results of the petri dish trials indicate that the migration of bacteria added to butter from agar or milk cultures is uncommon but may occur occasionally, especially in poorly worked butter.

Glass cylinder trials. In the glass cylinder trials the butter was packed into a cylinder as solidly as possible, enough space being left at each end of the cylinder so that a cotton stopper or cork could be inserted. The depth of the butter varied with the size of the cylinder; with small cylinders the depth was approximately two inches while with larger ones it was approximately four inches. One end of the mass of butter was inoculated over a small area next to the glass, care being taken that the organisms were definitely brought into contact with the glass. The butter was cultured principally at the other end of the mass, both next to the glass and away from it.

The cultures taken along the wall at the end opposite the one inoculated frequently showed the added organisms but in some instances were negative; in a number of the trials the bacteria were found in rather large numbers. Cultures taken 0.25 inch from the wall at the end opposite the one inoculated were regularly negative, even when considerable numbers of organisms were secured along the wall. The migration along the wall of a cylinder occurred with both motile and non-motile species.

From the results of the glass cylinder trials it is evident that bacteria may migrate extensively along the wall of a container in which butter is packed.

Trials with prints of butter. A few trials were carried out with prints of butter wrapped in parchment. The butter was inoculated by folding back the parchment, adding the culture and then replacing the paper.

When the surface of the butter was dry at the time of inoculation, or dried out very quickly, the added organisms could not be secured outside the inoculated area, even if the cultures were taken within 0.25 inch of it. In the trials in which moisture was held between the butter and paper for some days after the inoculation, cultures taken at a considerable distance from the inoculated area sometimes showed the added organism; the greatest distance an organism was found to have migrated was about two inches. Migration occurred with both motile and non-motile species. The importance of moisture along the surface of butter, from the standpoint of the movement of bacteria, was clearly shown in a few trials in which moisture extended in one direction from the inoculated area but not in another; where moisture was present the added organism commonly could be secured at a considerable distance from the inoculation, while where no moisture was present it could not.

The data obtained in the trials with prints of butter suggest that organisms may migrate along the surface of butter when it is kept moist with a parchment but do not when it is dry.

Trials in which butter in a jar was covered with parchment. The results secured with prints of butter suggested trials in which butter, in small jars, was inoculated at the center of the surface and then covered with parchment. Each trial involved two jars of butter, a dry parchment being used for one of them and a wet parchment for the other.

Cultures showed that with the dry parchment the organisms were not present outside the area of inoculation, while with the wet parchment they were present out to the edge of the parchment in all the trials. Migration of the organisms occurred with both motile and non-motile species.

The trials in which butter in a jar was covered with parchment indicate that bacteria may migrate along the surface when the paper is moist.

Trials in which butter in a jar was inoculated through a test tube imbedded in it. In several instances a test tube was imbedded in a jar of freshly churned butter so that it reached nearly to the bottom of the jar. The bottom of the tube was then broken with a glass rod and material from an agar or milk culture was mixed with the butter through the opening in the tube, an attempt being made to get the organisms next to the bottom of the jar. Cultures were taken only at the surface of the butter.

In a number of trials the inoculated organism was secured at the surface of the butter in cultures taken along the wall of the test tube but only when the test tube was firmly in contact with the butter. Frequently a space developed rather quickly between the butter and the test tube, due presumably to the loss of water from the butter, and in these instances the organism was not secured at the surface. When the organism was secured along the wall of the tube, it could not be secured 0.25 inch from the wall. The organism inoculated was never obtained at the surface of the butter along the wall of the jar.

The results of the trials in which butter in a jar was inoculated near the bottom through a test tube imbedded in it show that bacteria may migrate to the surface along the wall of the tube, provided the tube is firmly in contact with the butter.

(b) TRIALS IN WHICH A SMALL PORTION OF THE BUTTER WAS INOCULATED BY ADDING THE ORGANISM TO THE CREAM FROM WHICH IT WAS CHURNED

In the trials in which a small portion of the butter was inoculated by adding an organism to the cream from which it was churned, the inoculated

butter was placed in contact with the uninoculated in various ways. The inoculated butter commonly showed large numbers of the organism added to the cream although, occasionally, with long holding periods the numbers were comparatively small.

Contact through a small opening in a glass plate. The glass plates were about 3 x 4 inches and the opening in each was round with a diameter of 0.6 inch. After sterilization of a plate and the other equipment necessary to complete a set-up, inoculated butter was packed on one side of the plate and uninoculated butter on the other, the two being in contact through the opening in the plate. In some of the trials the plate was placed on edge in a beaker covered with a petri dish; there was a small amount of moist cotton in the bottom of the beaker. Since this arrangement does not exclude direct contact between the two pieces of butter through the air, a somewhat different set-up was also used. A half-pint milk bottle was cut into two parts about 1.5 inches from the bottom and the cut surfaces ground smooth with emery dust on a flat piece of glass. The glass plate with the butter in position was so placed that the inoculated butter was in the lower half of the bottle and the uninoculated butter was then covered with the upper part of the bottle. The parts of the bottle were sealed to the plate with vaseline or sealing wax. Moist cotton was used in the bottom of the bottle, the mouth of the bottle was cotton stoppered, and the cotton was covered with a beaker.

The added organism was secured from the uninoculated butter in some of the trials but not in others. The set-ups were not satisfactory because the exposure of the butter during the time it was being put in position on the plates rather frequently resulted in contamination with molds and it appeared that penetration of the butter by the mycelium favored the migration of bacteria through the butter. The growth of molds on the butter in the beakers and on the inoculated butter in the bottles undoubtedly was favored by the high humidity resulting from the moist cotton.

Contact through a small opening in a parchment. Contact through an opening in a parchment was arranged by packing the bottom of a petri dish full of inoculated butter, covering this with a piece of parchment through which a round hole about 0.5 inch in diameter had been cut, and then inverting another petri dish bottom that had been packed full of uninoculated butter over the first. Both the inoculated and uninoculated butter were firmly against the paper. The uninoculated butter was examined by lifting the dish containing it and making cultures from various points which were selected so that the organism could not have gotten to them by direct contact with the inoculated butter.

The cultures of the uninoculated butter regularly failed to show the organism added to the cream from which the inoculated butter was churned.

In the trials involving the contact of inoculated and uninoculated butter through an opening in a parchment, no evidence of the migration of bacteria to the uninoculated butter was secured.

Contact in prints. A print of uninoculated butter was prepared and then a layer of inoculated butter put over one end. The preparation was kept wrapped in a dry parchment.

The cultures of the uninoculated butter were negative, even when they were taken within 0.25 inch of the inoculated butter.

The trials in which inoculated and uninoculated butter were in contact in prints indicate that there was no migration of bacteria from the inoculated butter to the uninoculated.

Contact in cylinders or jars. A layer of inoculated butter about two inches thick was put into a cylinder or jar and then covered with a layer of uninoculated butter from about two to about four inches thick; with the cylinders the uninoculated butter was added through the end not used for the inoculated butter in order to exclude the possibility of contaminating the uninoculated butter with inoculated butter accidentally left on the wall of the cylinder. The uninoculated butter was cultured at the surface, both next to the wall of the container and away from it.

In most of the trials the organism in the inoculated butter was not found in the uninoculated butter; however, in two instances, one with a cylinder and one with a jar, the added organism was secured from the uninoculated butter along the wall of the container but not away from it.

The results of the trials in which inoculated and uninoculated butter were in contact in glass cylinders or jars suggest that bacteria do not migrate through butter but may migrate along the wall of the container.

Contact in petri dishes or jars. Petri dishes or jars were filled with the uninoculated butter, a hole about one inch in diameter was made with a sterile cork borer down through the butter near the center of the container, and the hole packed full of inoculated butter, care being taken that the butter did not touch the cover of the dish or jar. In a few trials the covers were left off the prepared petri dishes and some of the dishes held in a humidior while the others were held in a glass container that did not have any provision for keeping the moisture content of the air high.

The cultures of the uninoculated butter regularly failed to show the organism present in the inoculated butter, even when they were taken within 0.25 inch of the inoculated material. Holding uncovered dishes of the butter in a humidior had no effect on the results obtained.

In the trials in which inoculated and uninoculated butter were in contact in petri dishes or jars, no evidence of the migration of bacteria to the uninoculated butter was secured.

DISCUSSION OF RESULTS

The butter studied was unusually satisfactory for the growth of bacteria since it was made without salt and was held at temperatures that were suitable for the growth of many of the organisms common in dairy products. The failure of bacteria to migrate regularly through butter under these conditions indicates that butter offers considerable resistance to penetration by bacteria. Since bacteria migrated so readily through water held at the surface of butter by a parchment, it is evident that when a film or channel of water is formed the migration of bacteria is greatly favored. The migration of bacteria along the walls of containers presumably was due to the collection of water there, and in the few instances in which migration through butter occurred, water channels probably were involved.

Presumably, poorly worked butter contains more water channels than well worked butter and this probably explains the more frequent migration of bacteria through poorly worked butter in the few instances in which migration occurred. Because of the rapid migration of bacteria through water, the workmanship of butter evidently has an influence on the move-

ment of organisms through this product. King (2) has emphasized that such factors as washing, salting and especially working influence the collection of water in butter. This investigator has also pointed out that the water in butter comes from various sources, such as the cream, the water used for washing the butter, and the water added in establishing the desired composition; the source of water collecting at different points in butter may also be a factor in the migration of organisms because of the variations in the nutrients contained.

The migration of bacteria through butter is undoubtedly favored by the development of molds in the product. The growth of the mycelium through the butter opens up channels that favor the collection of water and these in turn favor the migration of bacteria. The effect of mold growth on the flavor and aroma of butter is usually so pronounced that it would ordinarily overshadow the action of bacteria.

The general results obtained indicate that unless there is water at the surface of butter the action of organisms with which butter is contaminated is largely limited to the points at which they are added. Apparently extensive bacteriological deterioration throughout a piece of butter is not likely to be caused by contamination of the finished butter but follows a wide distribution of organisms through the butter as a result of their presence in the cream. In this connection it should be recognized that the organisms coming from a churn are added to the cream primarily and that some of them are then retained in the butter; the strain thrown on a churn at the time butter is worked, however, may result in organisms being forced from cracks and crevices in which they were previously protected.

The migration of bacteria through water held between butter and a parchment shows that these organisms may sometimes spread over the surface of butter. Undoubtedly a comparable migration may occur between butter and a tub liner. Such a migration of organisms may be a factor in the development of objectionable flavors at the surface of butter.

It should be recognized that without any migration of bacteria there may be a large increase in the numbers per cubic centimeter of butter. The organisms that encounter favorable conditions may multiply and the descendants remain in the portion of water in which the growth began. Nelson and Hammer (3) noted the development of unusually long chains of butter culture organisms in unsalted butter held at temperatures favorable for growth, and it seems probable that the long chains were due to the organisms being held in the small portions of moisture that supported the growth.

The results obtained in the studies of the migration of bacteria through butter are of significance in a consideration of the physical constitution of butter. The failure of the organisms to migrate regularly in unsalted butter held at favorable temperatures suggests that water is not the continuous phase. Unpublished results secured at the Iowa Agricultural Experiment Station show that both motile and non-motile organisms, which grow well in milk, readily migrate through very rich cream so that when water is the continuous phase migration occurs even with a very high fat content. The observations on the migration of bacteria in butter are of interest in connection with the observations of Rahn and Boysen (4), who concluded that a considerable portion of the moisture of cream becomes sterile during churning by being divided into tiny droplets, and also the results of Hammer and Hussong (1) in which it was noted that in unsalted butter held at either 7° or 21°C. the multiplication of bacteria was more

rapid when the serum was separated from the fat than when the butter was in a normal physical condition.

SUMMARY

In the trials carried out, the migration of bacteria through unsalted butter, held at temperatures favorable for growth, was uncommon. This was the case both when the organisms were added from an agar or milk culture and when butter, that had been inoculated by adding organisms to the cream from which it was churned, was placed in contact with uninoculated butter. In the few instances in which migration occurred, poorly worked butter was involved more frequently than well worked butter. The distances bacteria migrated through butter were always comparatively small. Bacteria sometimes migrated extensively along the wall of a container in which butter was packed and also through water held at the surface of butter by a parchment.

Because of the comparatively rapid migration of organisms through water at the surface of the butter, it seems probable (a) that extensive migration along the wall of a container of butter was due to the collection of water there, and (b) that migration through butter was largely or entirely due to water channels in the product.

Poorly worked butter presumably contains more water channels than well worked butter and also more often permits the collection of water at the surface, so that the workmanship of butter is apparently a factor in the migration of bacteria through it or along its surface.

It should be noted that the trials involved unsalted butter held at temperatures comparatively favorable for the growth of the bacteria added. When there is little or no growth of bacteria in butter, because of the salt concentration or the holding temperature, the migration of bacteria through butter or along the surface is improbable.

The failure of various bacteria to migrate regularly through unsalted butter held at temperatures favorable for growth is of interest from the standpoint of a consideration of the physical constitution of butter. It definitely suggests that water is not the continuous phase since various bacteria readily migrate through cream having a very high fat content.

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THE PHYSICAL-CHEMICAL PROPERTIES OF ALCOHOL-GASOLINE BLENDS

I. INFLUENCE OF ALCOHOL CONCENTRATION AND OF TEMPERATURE UPON THE WATER-HOLDING CAPACITY

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The interest in the utilization of alcohol-gasoline blends as motor fuel has raised many questions as to the physical-chemical properties of such blends, as well as to the value of the blends as a fuel. Among the questions raised is that of the water-holding capacity of blends of gasoline with various proportions of anhydrous alcohol. There have been reported no difficulties in separation due to water absorption during extensive use in Sweden and Germany when temperatures reach low levels. However, the concentrations of alcohol used in these blends is from 20-30 per cent. It is necessary to know accurately the water-holding capacity of alcohol-gasoline blends for lower concentrations of alcohol. The data in the literature available do not deal with alcohol concentrations less than 10 per cent. This communication deals with data on the water-holding capacity of alcohol-gasoline blends containing as little as 1 per cent alcohol.

Preliminary studies indicated that the water-holding capacity of alcohol-gasoline blends is a function of the alcohol content, the temperature, and the character of the gasoline. The data given by Hubendick (1925) show the influence of these factors. Of the three factors, the character of the gasoline is of the least importance. It is supposed that the presence of unsaturated or aromatic constituents in the gasoline enhances its ability to mix with aqueous alcohol, that is, increases the water-holding capacity. Supposedly the worst gasoline, from the standpoint of miscibility with aqueous alcohol is one consisting mainly of straight chain saturated hydrocarbons.

In the work here reported, one gasoline was used, the properties of which are described later. Work is now in progress upon the influence of the composition of the gasoline and upon the influence of materials, such as the higher alcohols, upon the water-holding capacity of alcohol-gasoline blends.

EXPERIMENTAL METHODS

The alcohol was dried over lime and carefully distilled. Freedom from water was shown by testing with potassium permanganate and with anhydrous copper sulfate. The gasoline used was a representative mid-continent gasoline purchased locally and dried over calcium chloride. The Hanus number was 0.43 and the sulfuric acid soluble fraction was 25 per cent. The initial boiling point was 100°F. and the final 400°F.

Four alcohol-water mixtures were prepared, the water content being 4, 3, 2, and 1 per cent and the fifth preparation was 100 per cent alcohol. Mixtures of these alcohols and the above gasoline were then prepared, these mixtures containing 1, 2, 4, 6, 8, 10, 15, 20, 30, and 50 per cent alcohol or alcohol plus water. Samples of these several alcohol blends were then cooled until clouding appeared and were then warmed slowly until clear. The temperature at which the blend showed the first trace of turbidity was

measured and these results are shown in table 1. It was observed that noticeable separation did not occur until the temperature was lowered 6 or 8 degrees below those recorded. The endpoint was usually quite sharp, especially when the alcohol content of the blend was more than 4 per cent.

Throughout this report all percentages are by volume. The data are given in table 1.

TABLE 1. *Water-holding capacity of alcohol-gasoline blends*

100 per cent Ethanol cc. 100 cc. blend	Water cc. 100 cc. blend	Gasoline cc. 100 cc. blend	Temperature at which turbidity appears C.°
1.00	Nil	99.00	Below -60
0.99	0.01	99.00	-30
0.98	0.02	99.00	+1
0.97	0.03	99.00	+8
0.96	0.04	99.00	+14
2.00	Nil	98.00	Below -60
1.98	0.02	98.00	-30
1.96	0.04	98.00	0
1.94	0.06	98.00	+19
1.92	0.08	98.00	+25
4.00	Nil	96.00	Below -60
3.96	0.04	96.00	-25
3.92	0.08	96.00	+3
3.88	0.12	96.00	+24
3.84	0.16	96.00	+26
6.00	Nil	94.00	Below -60
5.94	0.06	94.00	-26
5.88	0.12	94.00	+2
5.82	0.18	94.00	+23
5.76	0.24	94.00	+25
8.00	Nil	92.00	Below -60
7.92	0.08	92.00	-25
7.84	0.16	92.00	+1
7.76	0.24	92.00	+19
7.68	0.32	92.00	+28
10.00	Nil	90.00	Below -60
9.90	0.10	90.00	-24
9.80	0.20	90.00	-1
9.70	0.30	90.00	+18
9.60	0.40	90.00	+27
15.00	Nil	85.00	Below -60
14.85	0.15	85.00	-25
14.70	0.30	85.00	-4
14.55	0.45	85.00	+14
14.40	0.60	85.00	+24
20.00	Nil	80.00	Below -60
19.80	0.20	80.00	-30
19.60	0.40	80.00	-10
19.40	0.60	80.00	+8
19.20	0.80	80.00	+21
30.00	Nil	70.00	Below -60
29.70	0.30	70.00	-35
29.40	0.60	70.00	-14
29.10	0.90	70.00	+2
28.80	1.20	70.00	+17
50.00	Nil	50.00	Below -60
49.50	0.50	50.00	-46
49.00	1.00	50.00	-23
48.50	1.50	50.00	-6
48.00	2.00	50.00	-8

The data of table 1 were recalculated to the basis of anhydrous alcohol content. In analyzing these figures, it was found that the following relation holds:

$$(1) \log (W \times 10^2) = 1.27 \log a + \log b$$

in which W = cc. of water required per 100 cc. of the blend at alcohol concentration a to produce turbidity. $\log b$ = the $\log W$ for $a = 1$. The equation shows that at any temperature the water-holding capacity varies as the alcohol concentration to the 1.27 power.

The values of $\log b$ and b for various temperatures are given in table 2.

TABLE 2. *Values of $\log b$ (see eq. (1)) for various temperatures*

t°	$\log b$	b
-40	-0.48	0.33
-30	-0.26	0.55
-20	-0.12	0.76
-10	0.0	1.00
0	0.10	1.32
+10	0.22	1.66
+20	0.27	1.86
+25	0.30	2.00

A graph (figure 1) of b against t° gives a linear relationship such that, (2) $b = 0.027 t^\circ + 1.33$
combining equations (1) and (2) (3) $\log (W \times 10^2) = 1.27 \log a + \log (0.027 t^\circ + 1.33)$

The use of equation (3) permits the calculation of the water-holding capacity of any alcohol blend up to 50 per cent alcohol at temperatures from -40° to $+20^\circ$.

In table 3, are given data for the water-holding capacity of 1, 2, 5, 10, 15, and 20 per cent alcohol-gasoline blends for various temperatures, calculated by eq. (3).

TABLE 3. *Water-holding capacity of various alcohol-gasoline blends, calculated by eq. (3)*

Conc. alc.	1	2	5	10	15	20
$T^\circ \text{ C.}$						
-40	0.003	0.007	0.022	0.053	0.089	0.126
-30	0.005	0.012	0.042	0.100	0.170	0.240
-20	0.008	0.018	0.062	0.150	0.251	0.355
-10	0.011	0.024	0.081	0.195	0.331	0.468
± 0	0.013	0.030	0.102	0.245	0.417	0.589
+10	0.016	0.036	0.123	0.295	0.501	0.708
+20	0.019	0.043	0.145	0.347	0.589	0.832
+25	0.020	0.046	0.155	0.371	0.631	0.891

The data from table 3, are plotted on the graph in figure 1. The linear relationship is at once evident, that is,

$$(4) W = nt^\circ + q$$

The values of n and q for various temperatures are given in table 4.

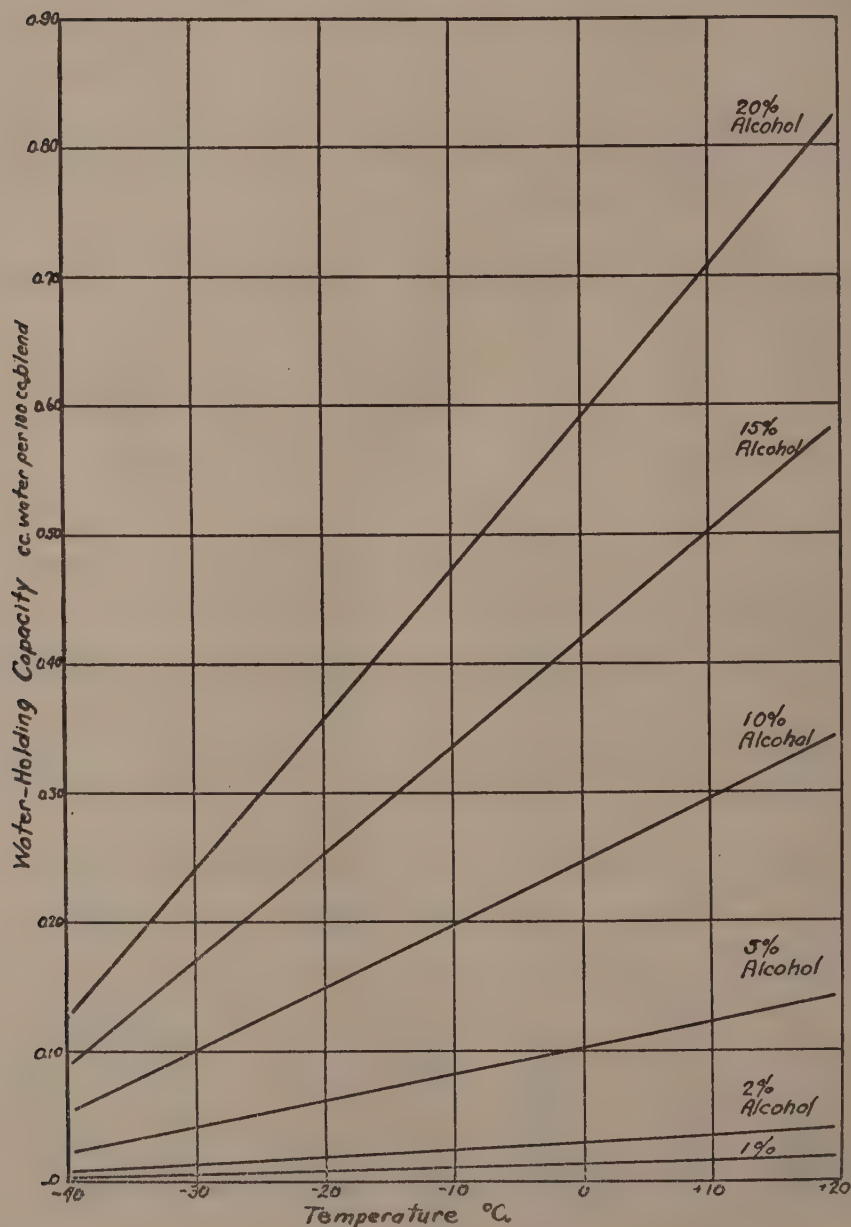


Fig. 1. The water-holding capacity of alcohol blends.

TABLE 4. *Values of n and of q for eq. (4)*

Conc. alcohol	$n \times 10^2$	$q \times 10^2$
1	0.0263	0.013
2	0.063	0.030
5	0.205	0.102
10	0.490	0.245
15	0.833	0.417
20	1.18	0.589

AN ANALYSIS OF DATA OBTAINED BY PREVIOUS AUTHORS

Hubendick, (1925). The data show the following relationship,

$$(5) \log (W \times 10^2) = 1.43 \log a = \log (0.0120 t^\circ + 0.60)$$

That is, while the water-holding capacity increases more rapidly with alcohol content than for our data, the value of $\log b$ is less and the water-holding capacity does not increase so rapidly with rise in temperature. While the water-holding capacity is less at higher temperature, the difference is slight at low temperatures.

It is evident that the values of n and $\log b$ in the equation

$$(6) \log (W \times 10^2) = n \log a + \log b$$

will prove valuable in characterizing various gasolines as to water-holding capacity with alcohol and also with various blending agents. This author cites experiments by Ekstrom for various gasolines. Curves are presented for -30°C showing a division of gasolines into two groups, one group stable at 98.1—98.3 per cent alcohol and the other group stable at 98.6—98.7 per cent. The gasoline used by us belongs to the latter group.

Sparrow, (1925) These data for 15 per cent alcohol at temperatures of -40° to $+20^\circ$ are identical with those obtained by us. He used an aviation gasoline which had met all government specifications.

Spausta, 1932. These data are for 20° only. His values correspond exactly with those obtained by us through the range of alcohol concentrations coincident with ours.

SUMMARY AND CONCLUSIONS

The water-holding capacity of alcohol-gasoline blends has been determined for a wide range of temperatures. A general relationship has been formed between the water-holding capacity and concentration of alcohol as,

$$\log (W \times 10^2) = m \log a + \log (nt^\circ + q) = m \log a + \log b.$$

The values of m and $\log b$ are characteristic of a given gasoline. For a given concentration of alcohol, the water-holding capacity is a linear function of the temperature.

Reports of the water accumulation in bulk storage tanks both in Iowa and in other states in the course of a year, have never exceeded 10 gallons of water for a 10,000 gallon tank. In larger tanks, of about 50,000 gallons capacity, accumulations have been claimed of 20 gallons per year. It is safe to assume that such tanks are emptied and filled ten times per year.

That is, the water absorbed will be about 0.01 per cent by volume of the fuel which passes thru them. This amount of water will raise the separation temperature of a 10 per cent blend about 2°C. Such a change is of very little significance.

In this consideration it has been assumed that the blend will absorb water no more rapidly than will gasoline. This should be practically the case, since the water accumulation results from tank breathing and condensation of the moisture thus introduced into the tank. Experiments are now in progress to test this assumption and will be reported in a later paper. If such alcohol blends are allowed to stand for one year they might absorb 0.1 per cent of water. This would raise the separation temperature of a 10 per cent blend about 20°, of a 5 per cent blend about 35° and of a 2 per cent blend over 120°. Such long storage is of course quite unusual. Blends prepared with commercial gasoline and commercial anhydrous alcohol show separation temperatures of —50 to —60°C. due to small amounts of water found in the gasoline and in the alcohol. It is evident from the above consideration, that a 10 per cent blend will remain miscible at the lowest winter temperatures even if stored for as long as one year. On the other hand, storage of 2-5 per cent blends should be limited to about one month in severe winter weather.

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CHANGES IN THE PECTIC CONSTITUENTS OF TOMATOES IN STORAGE¹

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The study of pectins was initiated by Braconnot (4) in 1825, when he published the description of a gelatinous substance which he had discovered in vegetable tissue and called pectic acid. Following this, many investigators became interested in Braconnot's gelatinous substance and a large amount of work was soon published.

Pectins are described by Carré (6) as "gelatinous colloidal substances of carbohydrate nature, occurring as a constituent of the cell-wall of plant tissue, particularly of fruits and certain roots such as turnips and carrots." They are classified with the group of substances known as the hydrophilic colloids and are assigned the role of a cementing material between the cell-walls of plant tissue.

Within recent years the significance of the pectic compounds in relation to the processes of plant life have been widely studied. As a result of studies of the pectins occurring in several fruits, Emmett (10), Carré (6), Norris (18) and others have established the fact that there is an association between the rate of pectin decomposition and the keeping qualities of fruits. It has been pointed out that there is a normal decomposition of pectin taking place within fruit from the time it ripens until its final breakdown. The rate of this decomposition has been found to be directly related to temperature.

Thus the rate of pectic decomposition within fruit has come to be regarded as an indicator of the metabolic activity and as a result of the longevity of the fruit. Therefore, a knowledge of the rate of pectic change in fruit is of fundamental importance in storage studies. In considering the problem of the storage of fresh tomatoes, then, the question naturally arises as to whether or not the pectic substances of the tomato behave as do the pectins of other fruits. What effect does temperature have on the pectins of the tomato, and just how much relation is there between the pectic changes and the keeping qualities of tomatoes? It is the purpose of this work to follow the pectic changes of tomatoes held at different storage temperatures in an effort to answer some of these questions, and to obtain some fundamental knowledge which may be of value in future studies of methods whereby the storage period of fresh tomatoes might be prolonged. Such a study is desirable, since no work of this type has been reported on tomatoes.

REVIEW OF LITERATURE

Much of the early work on pectins is confusing, because it deals with their chemical composition and technique whereby they might be extracted

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from plant tissue for comparative determinations. Since Branfoot (5) gives a complete bibliography and critical abstract of all the work done on pectins previous to 1929, an extensive review will be given here.

The first extensive work on pectins was published by Fremy (11). He described eight separate pectic compounds, and was the first to describe an insoluble pectin which he called pectose, furthermore he believed pectose to be the parent of the soluble forms. He considered that during the ripening of fruit pectose was decomposed to the soluble pectins by enzymes and acids of the cell-sap. Later, however, Bourquelot and Hérissé (3) attributed this transformation entirely to the action of enzymes.

The next discoveries of importance, as far as this work is concerned, were made by Mangin (16). He found from critical studies of the pectin of plant tissue that there were but three types of pectin that could be isolated for comparative determinations. Mangin (16) agreed with Fremy (11), however, on the hypothesis that the production of pectin was an important process in the ripening of fruits and that pectose was the parent substance of the soluble pectins. He also stated that pectose was intimately associated with the cellulose of the cell-walls and that the middle lamellae was composed of a pectic substance in the form of a calcium or potassium salt.

Carré (7) published the results of the first extensive work done on the pectic changes which occur in stored fruit. She found that the soluble pectin of apples increased with a corresponding decrease in pectose, up to three months in storage. After this time there occurred a decrease in all forms of pectin, which ended in the complete absence of pectin in the last stages of breakdown. It was found, however, that lower temperatures slowed up pectic decomposition and delayed from five to eight weeks the final breakdown. As had Fremy (11) and Mangin (16), Carré (7) concluded that the ripening of fruit was associated with the development of soluble pectin in the juice. At the time of picking the amount of soluble pectin in apples was negligible, but as ripening proceeded the soluble pectin increased and the pectose decreased. This process continued until the apples had reached the overripe or mealy condition, when a decrease in all forms of pectin began.

Further work enabled Carré and Horne (9) to confirm the chemical results with microscopical studies. These workers were able to distinguish the pectic structure of prepared mounts of apple tissue. A uniform layer of pectic material was found surrounding the cells of the apple from the time the fruit "set" until it reached its full size and the ripening processes began. This layer was thick while the apples were small but it became thinner as the fruit increased in size. When ripening began the pectic layer broke up into crescent shaped bodies, which became fewer and more disintegrated as ripening continued in storage and which completely disappeared in the last stages of senescence.

Sucharipa (19) reported work in which he was able to identify pectin in the juice and cell-walls of the lemon. He stated that the three major types of pectin occurred in layers in conjunction with the cellulose of the cell-walls, but that there was no clear-cut line of demarcation between the various pectic layers.

From studies of the pectins of oranges, Norris (18) reported that there was no difference in the pectin extracted from the cell-walls and that found in the juice. He attributed the presence of pectin in the juice to enzymes

which hydrolyzed it from that of the cell-walls. He stated that maceration also increased the amount of pectin in the juice.

Appleman and Conrad (1), working with peaches, were unable to identify pectic acid. However, they found a variation in soluble pectin corresponding to that found by Carré (7) in apples. Only slight changes were noted in the total pectic constituents of peaches until the fruit had reached the overripe stage. From this time until it completely disappeared in the final stages of senescence, there occurred a gradual decrease in pectin. As in the case of Carré (7) with apples, Appleman and Conrad (1) found the rate of pectic change within peaches to be directly associated with temperature. The lower temperatures gave the slower pectic changes.

Following their work on peaches Appleman and Conrad (2) studied the pectins of tomatoes in relation to the canned product. They found pectose predominating in the green fruit, with a partial hydrolysis into soluble pectin occurring as ripening advanced and a rapid transformation taking place during the latter stages of ripening when tomatoes are harvested for canning. A further conversion of insoluble to soluble pectin took place during the processing. A correlation was found between the ratio of pectose to pectin in the fresh fruit and the amount of distintegration of tomatoes in the can. Considerable variation in the percentage of pectin was found in the fruit of the various pickings, but little variation was found between varieties providing the fruit was harvested at the same time. A slight but consistent increase in the acidity of fresh tomatoes was noted as the season advanced, and also a corresponding increase in the rate of pectic decomposition.

From studies of ripe tomatoes under different storage temperatures, MacGillivray (15) found that the fruit lost volume and weight from the time it was removed from the vine. He noted also, losses in sugars, acids and organic constituents. The storage temperatures used were 13.9°, 27°, and 43°C. The higher temperatures gave the more rapid loss of the items listed above.

After working with the pectic constituents of apples, Haller (13) concluded that the softening of the fruit on the tree could not be entirely accounted for by changes in the pectic constituents. Soluble pectin, which was found only in small amounts while the fruit remained on the tree, stayed practically constant. The softening, however, was accompanied by a decrease in the amount of insoluble pectin. Agreeing with Carré (7), Haller (13) found an association between the softening of the fruit in storage and an accumulation of soluble pectin. The rate of softening of the apples at the various temperatures was reported as proportional to the rate of pectic decomposition.

From investigations of the chemical changes occurring in pears stored at different temperatures, Emmett (10) secured much the same results on the pectic changes as had Carré (7) from her apple storage studies. Marked differences were noted in the ripening rate of pears held at different temperatures. At 1°C. very little ripening of the fruit had occurred after six months in storage, while at 12°C. full ripeness was obtained in from 10 to 12 days. These differences were accompanied by similar variations in the rate of pectic change. The increase of soluble pectin at 1°C. was very slight, while at 12°C. the increase of soluble and the decrease of insoluble pectin indicated that the softening of the fruit was associated with the accumulation of soluble pectin. From his studies of the acidity of pears,

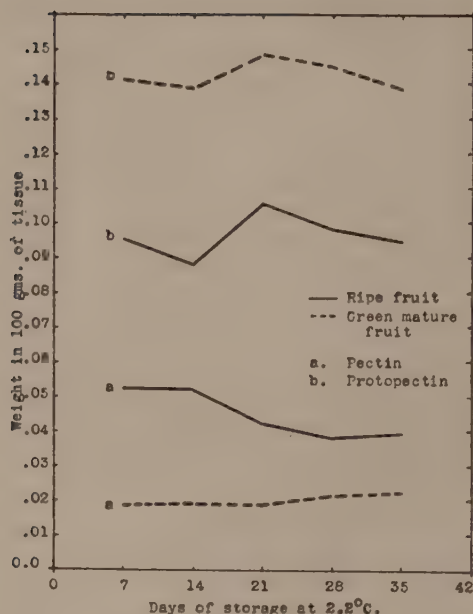


Fig. 1. Changes in pectin content of ripe and "green mature" fruit occurring in storage at 2.2°C.

seemed desirable to include in this study fruit at different degrees of maturity. Thus the tomatoes were divided into three classes, based on ripeness: (1) ripe fruit fully mature and in prime eating condition, (2) green-ripe fruit full size but still green in color, except for a slight pink appearing at the blossom end, and (3) green fruit full sized but still green in color with no pink showing anywhere. The fruit in the latter class proved difficult to select, so the 1931 sampling included only the first two classes of fruit.

The tomatoes were harvested in large lots and divided into the classes mentioned above. Enough fruit of each class was placed in each of the storage temperatures to allow the taking of five samples for pectin analysis. The storage period extended over an interval of 42 days.

SAMPLING

In harvesting the tomatoes, only fruits of medium size were selected, weighing from 160 to 200 grams. Seven tomatoes were allowed per sample for pectin analysis. The first sample was taken directly from the vine and the others at seven day intervals from the time the fruit was placed in storage.

The Globe variety of tomatoes was used for these determinations since it is the variety most widely used for forcing. Samples for storage and analysis were taken from plants grown in the greenhouse in the fall of 1930 and again in the fall of 1931. Results secured in 1931 were similar to those secured in 1930, so the figures given in the following tables are those for

Emmett (10) believed that the pectic changes were due to the presence of acid in the fruit as well as to enzymes.

Hopkins and Gourley (14) discovered no correlation between fertilizer treatments and the percentage of pectin in grapes. These workers noted, however, a change of insoluble to soluble pectin as the grapes ripened.

PROCEDURE

From studies and observations, Haber (12) noted that tomatoes kept more satisfactorily at temperatures above those used for pome fruits. As a consequence the temperatures selected for this work were 2.2°C. (36°F.), 10°C. (50°F.) and 21.1°C. (70°F.)

Since it is common practice among shippers to harvest their fruit green, it

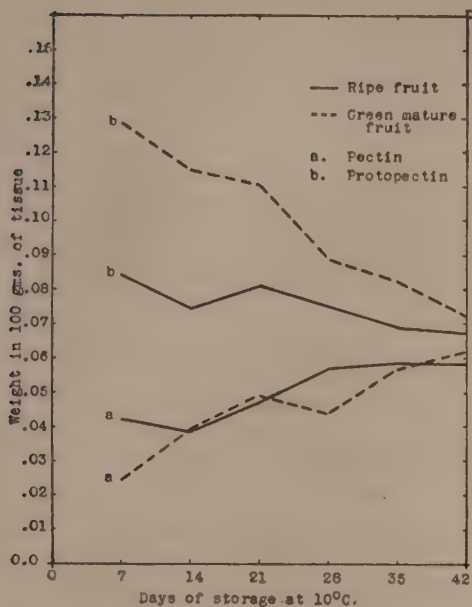


Fig. 2. Changes in pectin content of ripe and "green mature" fruit occurring in storage at 10°C.

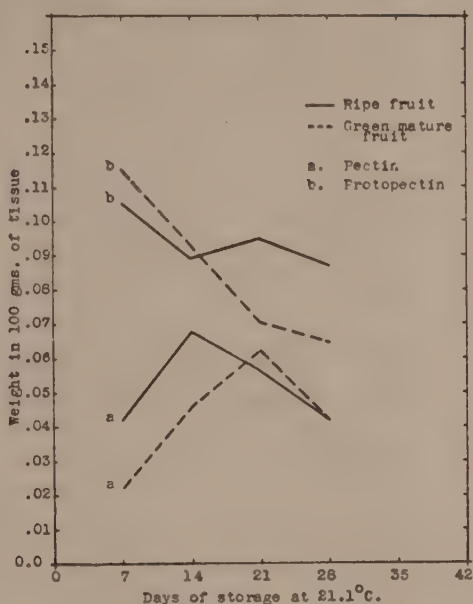


Fig. 3. Changes in pectin content of ripe and "green mature" fruit occurring in storage at 21.1°C.

the 1931 crop. Closer checks were secured in 1931 than in 1930 because of better laboratory technique.

METHOD OF ANALYSIS

Nanji and Norman (17) describe means of determining three individual pectic substances: (1) a soluble pectin extracted with distilled water, (2) an insoluble pectin (pectose) extracted with 0.5 per cent oxalic acid, and (3) a second insoluble pectin (pectic acid) extracted with 0.5 per cent ammonium oxalate. Check results could not be obtained with this method of analysis and it was discarded. Check analysis were secured with the method of Appleman and Conrad (2) for the determination of pectin and protopectin in fresh tomatoes. Their method is essentially the same as that of Carré and Haynes (8) with some slight modifications. They found that the calcium pectate gel obtained from samples of fresh tomatoes contained considerable material other than calcium pectate, so it was necessary to purify the gel. To the beaker containing the weighed calcium pectate and impurities were added 50 cc. of a two per cent solution of ammonium citrate and then boiled for 45 minutes to dissolve the calcium pectate. The residue obtained was dried to constant weight and this weight subtracted from the weight of the original calcium pectate gel.

RESULTS

The following tables and figures 1, 2, and 3 give the results of the soluble and insoluble pectin or, as Appleman and Conrad (2) stated, the pectin and protopectin.

TABLE 1. *Weight of pectin in terms of calcium pectate gel in 100 grams of fresh ripe fruit stored at 2.2°C.*

Length of storage period	Pectin	Protopectin	Total
7 days	.0520	.0954	.1474
14	.0518	.0879	.1397
21	.0422	.1053	.1475
28	.0378	.0976	.1354
35	.0398	.0942	.1340

TABLE 2. *Weight of pectin in terms of calcium pectate gel in 100 grams of fresh "green mature" fruit stored at 2.2°C.*

Length of storage period	Pectin	Protopectin	Total
7 days	.0180	.1412	.1592
14	.0192	.1392	.1494
21	.0187	.1482	.1569
28	.0218	.1453	.1671
35	.0222	.1380	.1602

TABLE 3. *Weight of pectin in terms of calcium pectate gel in 100 grams of fresh ripe fruit stored at 21.1 C.*

Length of storage period*	Pectin	Protopectin	Total
7 days	.0418	.1053	.1471
14	.0673	.0895	.1568
21	.0564	.0942	.1506
28	.0423	.0865	.1288

TABLE 4. *Weight of pectin in terms of calcium pectate gel in 100 grams of fresh "green mature" fruit stored at 21.1°C.*

Length of storage period*	Pectin	Protopectin	Total
7 days	.0214	.1153	.1367
14	.0458	.0932	.1390
21	.0626	.0701	.1327
28	.0422	.0645	.1067

* Stored only 4 weeks because fruit decayed rapidly at this temperature.

TABLE 5. *Weight of pectin in terms of calcium pectate gel in 100 grams of fresh ripe fruit stored at 10°C.*

Length of storage period	Pectin	Protopectin	Total
7 days	.0418	.0838	.1256
14	.0382	.0742	.1124
21	.0468	.0808	.1276
28	.0562	.0749	.1311
35	.0587	.0692	.1379
42	.0582	.0668	.1250

TABLE 6. *Weight of pectin in terms of calcium pectate gel in 100 grams of fresh "green mature" fruit stored at 10°C.*

Length of storage period	Pectin	Protopectin	Total
7 days	.0242	.1278	.1520
14	.0395	.1145	.1540
21	.0482	.1101	.1583
28	.0434	.0878	.1312
35	.0564	.0823	.1387
42	.0617	.0721	.1338

The increase of soluble pectin and a decrease in insoluble or protopectin occurs most rapidly in fruits stored at 21.1°C. After the third week in storage at 21.1°C. the pectin as well as the protopectin, decreased, this might be expected since the fruits after this period were not in first-class condition. Fruits that were "green mature" when placed in storage at this temperature ripened in less than a week and were dead ripe in two weeks. After the third week decay started in many fruits and, although apparently only sound fruits were used for analyses, this probably accounts for the rapid decrease in total pectin.

At 10°C. green fruits ripen very slowly and the change in pectin is slow, although total pectin decrease slightly during the 42-day storage period. It seems logical that the insoluble pectin in the ripe fruit should change very slowly, since the tomatoes remain firm throughout the storage period. The changes in color of fruit are slight. The ripe tomatoes had taken on a slightly lighter tinge, and the mature green fruit color had faded to a greenish yellow with considerable pink spreading away from the calyx. The flesh of the ripe and mature green fruit had remained quite firm and of a good color, but the taste was flat, having lost the characteristic acid flavor of the tomato.

The smallest changes in pectins occurred in tomatoes stored at 2.2°C. This was true only when the fruits were held at this temperature because when removed from 2.2°C. to room temperature changes in pectins occurred very rapidly. The insoluble pectin tended to remain constant in both the green mature and ripe fruit, while the soluble pectin decreased slightly during the 35-day storage period in the ripe fruit. Little change occurred

in the green mature fruit while very little change was noted in the color of the green fruit and it remained quite solid to the end of the storage period of 35 days. The ripe fruits at the end of the fourth week showed a lighter shade of red than when placed in storage, although they remained firm. Since the fruits did remain firm, little change might be expected in the pectin.

DISCUSSION

The results obtained in this work agree with results obtained by other investigators from similar studies of the pectic substances of other types of fruit. This was true when Appleman and Conrad's (2) modification of the Carré-Haynes' (8) method was used.

An inter-related change of pectins from the insoluble to the soluble form was the normal process in the ripening of the fruit. Such a change caused the soluble pectin to accumulate at the expense of the insoluble form as the fruit matured. However, a decrease in the soluble and total pectin occurred at the highest storage temperature, 21.1°C.

From a study of the tables it will be noted that the rate of change in soluble and insoluble pectin varied directly with the storage temperature. Very little change in the pectin-protpectin ratio occurred at 2.2°C. The changes were greater at 10°C. and very rapid at 21.1°C.

Appleman and Conrad (2) reported an increase of soluble pectin as the tomato ripened. The tomato is a short lived fruit, and its metabolic activity is high so one might expect a decrease in soluble and total pectin over a fairly long storage period, especially at the higher temperatures. This concurs with the work of Carré (7), who reported that all pectic substances of apples decreased after a peak of pectic accumulation, about the time the fruit gained full ripeness.

From the practical point of view it was found that tomatoes remained in a better condition at 10°C. than they did at either 2.2°C. or 21.1°C., 2.2°C. proved too cool and 21.1°C. too warm for the keeping of fresh tomatoes. It seems, therefore, that the ideal temperature for the storage of tomatoes lies somewhere between 10°C. and 21.1°C. In a recent publication, Wright et al (20) found 10°C. fairly satisfactory but 12.8°C. was better and recommended the latter temperature for either storage or delayed-ripening purposes.

Although pectic changes are much slower at 2.2°C. than at 10°C., the latter storage temperature is recommended since the fruits do not break down so rapidly when removed to room temperature.

SUMMARY AND CONCLUSIONS

1. The percentage of soluble pectin increased as the fruit passed from the green to the ripe condition either on the vine or in storage.
2. The rate of increase from insoluble to soluble pectin varied directly with the temperature, the highest temperature giving the more rapid change.
3. Soluble pectin as well as insoluble pectin decreases if the storage period is prolonged, especially at higher temperatures.
4. The keeping qualities of the tomato do not seem to be wholly dependent upon the rate of pectic change, but softening of the fruit is accompanied by changes from the insoluble to the soluble form.

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INSECTICIDAL ACTION IN THE NITROGEN HETEROCYCLIC COMPOUNDS

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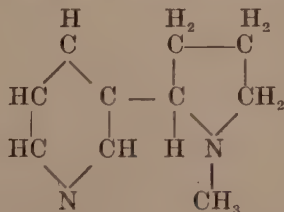
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Among insecticides, nicotine occupies a position of exceptional importance. Attempts to synthesize compounds in the N-heterocyclic series which duplicate or even closely approach it in toxicity to certain insects have been largely unsuccessful. The recent synthesis of neonicotine (14), and the discovery of its naturally occurring optical isomer, anabasine (11, 12, 15, 4) have, however, furnished two compounds in a related series which resemble it in insecticidal activity. Compared with several other important organic insecticides, notably the pyrethrins and rotenone, nicotine is a compound of rather simple structure; and it seems peculiar that other closely related compounds of value, besides those mentioned above, have not been discovered.

A number of investigations of the toxicity of N-heterocyclic compounds to insects have been published. LaForge (7, 8) synthesized, and Richardson and Shepard (13) studied the insecticidal action of a group of these compounds which are closely related to nicotine. Harlan (5), Tattersfield (17) and Tattersfield and Gimingham (18) have also made important contributions to this subject. The low toxicity of the related synthetic compounds which they examined convinced these authors that it is the make-up of the nicotine molecule as a whole rather than the presence of a specific toxic linkage or toxophore grouping which is responsible for the extreme toxicity. The attachment of the pyrrolidine nucleus at the β -position of the pyridine ring, and the presence of an asymmetric carbon atom have also been mentioned (17) as contributing to the toxicity of nicotine. More recent investigations with neonicotine (14) indicate, however, that a high degree of toxicity is possible in a pyridyl compound in which piperidine rather than methyl-pyrrolidine occupies the β -position. The similarity in structure, physical properties and insecticidal action of neonicotine and nicotine furnished an incentive for this further investigation of toxicity in the N-heterocyclic series.

Previous chemical studies of nicotine (2) have shown that the molecule is a very reactive one and that the reactive point in the molecule,



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is the carbon-nitrogen bond of the pyrrolidine nucleus adjacent to the pyridyl group. The weakness of this bond is apparently due to the extreme negativity of the pyridyl radical (3). This information suggested an investigation of the toxicity to insects of a series of α -substituted N-methylpyrrolidines in which the negativity of the substituting radical varied within wide limits. Unfortunately the difficulty of synthesis renders inaccessible the more desirable compounds, i.e. those in which an extremely negative radical occupies the alpha position on the pyrrolidine nucleus.

All members of the series investigated contain an asymmetric carbon atom and optical activity is therefore possible. Since resolution of the inactive compounds would be extremely difficult, the strongly laevorotatory nicotine was racemized, and the inactive form was compared with the other members of the series.

EXPERIMENTAL

The method of determining the relative toxicity of the compounds was essentially that described by Tattersfield and Morris (16) in which groups of the insects are uniformly sprayed in an inclosure with aqueous solutions or emulsions of the compounds. The test insect was the bean aphid, *Aphis rumicis* L., taken directly from one of its host plants, Nasturtium, grown in the greenhouse. Only the adult wingless forms were used in the tests.

The spray solutions or emulsions were usually made up to contain 0.25 per cent by weight of sodium oleate which served as a spreading agent. As α -(p-chlorophenyl)-N-methylpyrrolidine will not form a stable emulsion with 0.25 per cent soap, it was necessary to shake the mixture thoroughly before each application. The toxic value was also checked by doubling the soap concentration, the increased soap exerting a peculiar solvent effect which converted the emulsion into a true solution. All the compounds were oils except α -(p-hydroxyphenyl)-N-methylpyrrolidine which is a solid and therefore required a different treatment. It was first dissolved in a small volume of acetone and then diluted to volume with the calculated amount of soap solution. A more concentrated solution than that given could not be used as it tended to crystallize in the spray nozzle.

The spray preparations were delivered through a small atomizer nozzle at such a rate that 0.56 g. of liquid fell in 30 seconds on the dish that held the group of insects. This value was checked at intervals throughout the course of the experiments to insure its constancy. There were ten insects in each group, and five replications were made for each concentration of a compound. Whenever practical, sufficient concentrations were employed to indicate the trend of the toxicity curve.

After spraying, the aphids were immediately placed on fresh nasturtium leaves previously arranged in small bottles containing water to prevent wilting. The bottles were set in pans of water to preclude escape of the insects. The dead and living insects were counted after twenty-four hours.

All experiments were accompanied by controls which received a spray of 0.25 per cent sodium oleate solution alone or of sodium oleate and acetone of the same concentration employed in the several experiments². Previous

² Tattersfield and coworkers have used a 1 per cent saponin instead of soap as a spreading agent in their toxicity experiments. Our data for nicotine with 0.25 per cent sodium oleate are, however, in substantial agreement with their data from 3 year's experiments with nicotine and saponin (18). Other data, independently derived in this laboratory, confirm this agreement. Apparently a concentration of 0.25 per cent sodium oleate has no greater influence upon the toxicity of nicotine for adult wingless *Aphis rumicis* than does 1 per cent saponin, and it is a somewhat better spreading agent.

work under identical experimental conditions showed that this concentration of sodium oleate kills from about 16 to 20 per cent of the adult wingless aphids. As the results from the control groups were slightly variable in the different experiments, the actual percentage of mortality is not given but rather the net percentage of mortality, a term defined by the expression, $\frac{(X-Y)100}{X}$, where X is the per cent alive in the control group and Y the percent alive in the experimental group.

The results are given in table 1.

TABLE 1. *Toxicity data for some α -substituted N-methylpyrrolidines with *Aphis rumicis* as the test insect*

Compound	Concentration g. per 100 cc. ^a	Range in net p'ct'g Mortality ^b	Average net p'ct'g Mortality ^b
α -(β -Pyridyl)-N-methyl- pyrrolidine (<i>l</i> -nicotine)	0.1	73-100	87
	0.05	42-77	66
	0.033	16-69	43
	0.025	10-40	22
	0.02	10-20	14
	0.01	0-29	12
Racemic α -(β -Pyridyl)-N- methylpyrrolidine (<i>dl</i> -nicotine)	0.2	79-100	93
	0.134	55-77	66
	0.1	37-58	42
	0.1	40-70	51
	0.033	0-26	11
α -(<i>p</i> -Chlorophenyl)-N- methylpyrrolidine	1.0	88-100	97
	0.5	29-88	54
	0.5	75-100	92
	0.4	22-45	35
	0.33	0-60	30
	0.33	53-100	69
	0.33	0-53	16
	0.25	5-26	18
	0.25	11-44	31
	0.25	0-20	12
α -(<i>p</i> -Methoxyphenyl)-N- methylpyrrolidine	1.34	—	100
	1.0	68-100	83
	1.0	38-100	73
	0.8	37-90	61
	0.77	5-16	8
	0.66	0-40	26
	0.5	0-30	12
α -(<i>p</i> -Hydroxyphenyl)-N- methylpyrrolidine	1.0	0-11	2
α -Phenyl-N-methyl- pyrrolidine	4.0	—	100
	2.0	60-100	80
	1.33	25-87	49
	1.11	26-58	45
	1.0	0-20	6
	0.333	0-29	7
α - <i>n</i> -Butyl-N-methyl- pyrrolidine	3.33	—	100
	2.0	44-100	73
	2.0	26-58	41
	1.54	30-70	54
	1.33	0-77	39
	1.0	0-26	13

Table 1, continued

Compound	Concentration g. per 100 cc. ^a	Range in p'ct'g Mortality ^b	Average net p'ct'g Mortality ²
α -n-Propyl-N-methyl- pyrrolidine	5.0	—	92
	4.0	—	48
	4.0	76-100	83
	3.3	30-50	40
	3.3	0-17	7
	2.0	0-22	6
	1.33	0-46	8
α -Ethyl-N-methyl- pyrrolidine	5.0	0-11	2
	2.0	—	0
α -Methyl-N-methyl- pyrrolidine	7.1	0-20	9
	4.0	—	0
	2.0	—	0
N-Methylpyrrolidine	4.0	0-20	10

^a Time constant, 24 hours.^b Five groups of 10 insects each.TABLE 2. Comparative toxicity to *Aphis rumicis* and some physical constants of certain α -substituted N-methylpyrrolidines

Compound α -substitued-N- methylpyrrolidine	Approx. Con- centration in g. per 100 cc. for 50 percent Mortality	Dissociation Constant	Boiling Point °C.	Nature of spray liquid
β -Pyridyl-(l-nicotine)	0.04	9×10^{-7}	248	solution
Racemic β -Pyridyl- (dl-nicotine)	0.12	9×10^{-7}	248	solution
α -(p-Chlorophenyl)-	0.44	5×10^{-6}	118/9 mm.	emulsion
α -(p-Methoxyphenyl)-	0.8	8×10^{-6}	130/9 mm.	emulsion
α -(p-Hydroxyphenyl)-	>1.0		a	solution
α -Phenyl-	1.33	6×10^{-6}	217.5	emulsion
α -n-Butyl-	1.8	6×10^{-5}	170	emulsion
α -n-Propyl-	3.8	6×10^{-5}	147-8	emulsion
α -Ethyl-	>5.0	6×10^{-5}	122-4	solution
α -Methyl-	>7.1	6×10^{-5}	96-7	solution
α -Hydrogen-(N- methylpyrrolidine)	>4.0	1.5×10^{-4}	78	solution

^a A solid, melting point 157° C.

In table 2 the toxicity data are summarized and the dissociation constants and boiling points of the compounds and the nature of the spray liquid are included for comparison. The toxicity values are the approximate concentrations, in grams per 100 cc., which produce 50 per cent net mortality in 24 hours. They were estimated from smooth curves fitted by eye to the toxicity data. Experience has shown the 50 per cent points to

be the most valuable statistics for comparative purposes derivable from this type of toxicity data.

A study of the pharmacological action of these compounds is in progress in cooperation with Dr. D. I. Macht³ of the Hynson, Westcott and Dunning Laboratories at Baltimore, Maryland. The results are summarized in part in table 3 because of their comparative relation to the present work.

The toxicity values given in table 3 are based upon the time required to kill goldfish, *Carassius aureus*, and tadpoles of *Rana sylvatica* and to inhibit the growth of seedlings of *Lupinus albus*. The index of growth is the percentage of growth of the seedlings in the experimental solution compared with that made by seedlings in a nutrient solution, a method reported by Dr. Macht in numerous publications.

Other pharmacological investigations to date have included only a comparison of *l*-nicotine and α -phenyl-N-methylpyrrolidine. Both compounds produce a stimulation of uterine contractions, *l*-nicotine being much the more powerful. Neither compound affects the contractions produced on smooth muscle organs by a subsequent dose of epinephrine. Their effect upon the blood pressure and respiration has been studied by intravenous injection of cats under ether anesthesia. Although the toxicity is different, the lethal dose for *l*-nicotine being 2 mg. per Kg. of body weight, whereas that for α -phenyl-N-methylpyrrolidine is 12 mg., qualitatively the results are similar. Death in both cases was produced by primary paralysis of the respiration, secondary depression of the circulation, and standstill of the heart.

DISCUSSION

In the series of compounds studied the only variable from a structural standpoint is the α -substituent on the pyrrolidine nucleus. The change in basicity is the result almost entirely of the difference in negativity or relative affinity of each radical. This difference in the influence of the radical can best be expressed according to the theory of the "electron-sharing ability" of organic radicals of Hixon and Johns (6) which gives the different radicals a mathematical relationship.⁴

An examination of table 2 shows an apparent correlation between the dissociation constants or basicity of the compounds and toxicity. It is doubtful whether hydroxyl ion concentration or basicity in itself plays a major role as toxicity increases in the series as basicity decreases. Change in toxicity is more probably the result of some change in the reactivity of the molecule, which is correlated with the same influence that increases or decreases the basicity, and which is most conveniently called the "electron-sharing ability" of the radical.

³ The pharmacological studies will be reported elsewhere by Dr. Macht.

⁴ A negative radical is defined in terms of the tendency to take on an electron; similarly, a positive radical is one which gives up an electron. This "electronic potential" cannot be measured by any known method. On the assumption that this potential is transmitted to and causes the variation in any polar group attached to a molecule, various radicals can be arranged in the order of their influence upon the free energy of the polar group. Since mass, steric factors, and orientation, as well as electronic potential, must play a role in this effect of the radical upon the free energies of the polar group, the phenomenon has been designated by the inclusive term "electron-sharing ability". Any reversible reaction may be used as a measure of free energy; one of the most convenient measurements is that of the dissociation constants. For a more complete discussion of the term, "electron sharing ability", the reader is referred to the original work.

TABLE 3. Toxicity of certain α -substituted *N*-methylpyrrolidines to goldfish, tadpoles, and lupine seedlings

Compound	Time in Mins. to Kill Goldfish ^d		Time in Mins. to Kill Tadpoles ^e		Index of Growth of Seedlings ^f	
	Free Base ^a	Hydrochloride ^b	Free Base	Hydrochloride ^b	Free Base ^a	Hydrochloride ^b
<i>l</i> - α -(β -Pyridyl)- <i>N</i> -methylpyrrolidine (<i>l</i> -nicotine)	5	15	2 ^c	5	46	69
<i>dl</i> - α -(β -Pyridyl)- <i>N</i> -methylpyrrolidine (<i>dl</i> -Nicotine)	—	—	5 ^c	—	49	—
α -(<i>p</i> -Chlorophenyl)- <i>N</i> -methylpyrrolidine	—	40	12 ^a	30	—	81
α -Phenyl- <i>N</i> -methylpyrrolidine	12	47	5 ^a	18	40	73
α - <i>n</i> -Butyl- <i>N</i> -methylpyrrolidine	14	105	6 ^a	78	54	75
α - <i>n</i> -Propyl- <i>N</i> -methylpyrrolidine	15	155	7 ^a	1320	50	82
α -Ethyl- <i>N</i> -methylpyrrolidine	34	>1080	8 ^a	>4320	64	85
α -Methyl- <i>N</i> -methylpyrrolidine	68	>1080	12 ^a	3600	66	88
<i>N</i> -Methylpyrrolidine	70	>1080	9 ^a	>4560	63	91

^a Concentration 0.02 g. per 100 cc.^b Concentration 0.1 g. per 100 cc.^c Concentration 0.01 g. per 100 cc.^d *Carassius auratus*.^e *Rana sylvatica*. Tadpoles used in tests with the free bases were 2 weeks old, those used with the hydrochlorides were 1 week old.^f Percentage of growth of *Lupinus albus* seedlings compared to that of the seedlings in Shive's solution.

Such a correlation cannot be expected to hold quantitatively for each radical nor can a strictly linear relation be anticipated. Differences in physical properties which affect permeability may play an important role. Species resistance of the organism will no doubt be a disturbing factor in some cases. However, it is significant that the studies with other organisms place the compounds essentially in the same order of toxicity as the insecticidal studies.

A slight change in negativity of the substituting radical in the present series would not be apparent in the dissociation constant of the base determined by methods known at present as the influence must be transmitted through one carbon and one nitrogen atom. Therefore α -n-butyl- and α -n-propyl-N-methylpyrrolidine give substantially the same constants whereas, a slight difference exists in the negativity of the two α -substituents.

The toxic values for *l*-nicotine and *dl*-nicotine are of considerable interest aside from their relation to the values for other members of the series. If no toxic influence were exerted by the *dextro* half of the inactive nicotine, the concentration for 50 per cent mortality should be about 0.08 g. per 100 cc. instead of 0.12 g., the value actually found. Macht (9) has shown that *l*-nicotine exerts a synergistic influence on *dl*-nicotine in its toxicity to goldfish placed in a solution of the alkaloid, to rats by intraperitoneal injection, and to cats and rabbits by intravenous injection. In all cases toxicity greater than the additive effect of the two preparations was noted. In contrast to these effects on vertebrate animals, a nearly equal mixture of *dextro*- and *laevo*-nicotine⁵ applied to the body surface of aphids, exerts perhaps a slight antagonistic action. Certainly there is no evidence of a synergistic effect. However, these results are not strictly comparable to those reported by Macht as his studies were concerned with mixtures of *l*-nicotine and *dl*-nicotine which contained 62.5 per cent of *laevo*- and 37.5 per cent of *dextro*-nicotine.

A study of the insecticidal action of *d*-nicotine would be instructive. *d*-Nicotine has been reported by Mayor (10) to be one-half as toxic to dogs and guinea pigs as *l*-nicotine and its physiological action to be of a somewhat different nature.

A brief discussion of two of the recent insecticidal and pharmacological studies on anabasine and related compounds will not be out of place here. Campbell, Sullivan and Smith (1) give the following order of toxicity to *Culex* larvae (*C. pipiens*, *C. territans*, and *C. quinquefasciatus*) of a number of these compounds; Nicotine 100; anabasine 38; methyl anabasine 21. They report also the results of several investigators who found anabasine as toxic as nicotine to a certain species of aphids, a result similar to that previously reported for the optically inactive neonicotine (14). It is interesting to note that anabasine together with nor-nicotine (*dl*-[β -pyridyl]-pyrrolidine) has recently been isolated from tobacco by Ehrenstein (4) who also reports upon their pharmacological action. The effects of nicotine, nor-nicotine, anabasine and methyl anabasine (*dl*-[β -pyridyl]-N-methylpiperidine) upon the tonus of frog muscle, and upon the blood pressure and heart activity of the cat after intravenous injection were qualitatively similar but there were quantitative differences. On frog muscle the effect was manifested in the order: nicotine = methyl anabasine > anabasine > nor-nicotine. All the compounds mentioned were less active than nicotine on

⁵ The specific rotation of the *dl*-mixture was -2.71 ; as nicotine has a specific rotation of -169 , the content of *l*-nicotine of this mixture was about 1.6 per cent in excess.

the heart and on the blood pressure of the cat; anabesine and methyl anabesine, however, most nearly resembled nicotine in their action.

SUMMARY AND CONCLUSIONS

1. This is a study of the relative toxicity of a group of eleven α -substituted N-methylpyrrolidine compounds to the bean aphid, *Aphis rumicis*, in which the negativity of the substituted radicals as measured by the dissociation constants, varied within the limits of 10^{-4} and 10^{-7} . The toxicities of most of these compounds for the goldfish (*Carassius aureus*) and for tadpoles of *Rana sylvatica* and their effect on the growth of lupine seedlings, (*Lupinus albus*) are included for comparison.

2. The compounds were made up in aqueous solution with 0.25 per cent sodium oleate and administered as a fine spray to the adult wingless aphids.

3. Toxicity to the aphids, expressed as the concentration which produces 50 per cent mortality in 24 hours, is correlated with the dissociation constants of the compounds, decreasing as the values of the constants increase. Toxicity to the other organisms follows, in general, the same order.

4. The progressive decrease in toxicity is not the result of a change in hydroxyl ion concentration. Rather it is correlated with that influence of the substituent which alters the reactivity of the molecule and simultaneously results in a change of basicity. This influence is conveniently called the "electron-sharing ability" of the substituted radical.

5. The correlation between the electron-sharing ability and toxicity is not quantitative, neither does it show a strictly linear relation. Differences in the physical properties of the substituting radicals which govern permeability and differences in the "innate" resistance of species are disturbing factors. Slight differences in the negativity of compounds may not be apparent in the dissociation constants yet the relative toxicity may be different.

6. *l*-Nicotine is considerably more toxic to aphids than the optically inactive mixture (*dl*-nicotine).

7. The recent literature on the toxic action of anabesine (*dl*-(β -pyridyl)-N-piperidine) and its N-methyl derivative is discussed in relation to nicotine. These compounds appear to be less toxic to certain insects and less active physiologically to vertebrates than nicotine. They resemble neonicotine, their optically inactive isomer, in being about equally toxic with nicotine to aphids.

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STUDIES ON A COLOR DEFECT IN BUTTER¹

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Butter showing a pronounced pink color has recently been sent to the Dairy Industry Section of the Iowa Agricultural Experiment Station from two widely separated sources, with the request that the cause of the unusual color be determined. The results of the studies of the butter are reported herein.

CASE 1

A part of a quarter-pound print of butter was forwarded to the laboratory from one of the western states. The plant that had manufactured the butter shipped butter to various retail establishments but the pink color was reported through only one of them.

DESCRIPTION OF THE DEFECT

There were approximately twelve pink spots distributed over one end and about one-third of the length of the original print. The spots were irregular in size and shape with the long diameters varying from 1 mm. to 15 mm. Sections through the print showed that the pink spots were present only at the surface and penetrated only to a depth of about 1 mm. In some instances there appeared to be a wrinkle in the parchment wrapper immediately over a pink spot.

EXPERIMENTAL

The nature and distribution of the pink spots suggested that microorganisms might be responsible for them and, accordingly, microbiological examinations were carried out. Microscopic examinations indicated that the general flora of the pink areas was essentially the same as that of the normal butter. Plates poured with material taken from the pink areas, using various media and incubation conditions, showed that these areas did not contain unusual numbers or types of organisms.

Since microorganisms did not appear to be responsible for the defect, the possibility of it being caused by a chemical reaction involving the butter color was investigated. Slices of the butter were placed in petri dishes and treated with diacetyl and solutions of sodium chloride, potassium nitrate, sodium hypochlorite, hydrogen peroxide and potassium chlorate. None of these reagents caused a significant color change. At this point in the study it was reported that the butter showing the color defect had been stored in a mechanical refrigerator and, accordingly, a sample of the butter was placed in a large wide-mouthed bottle, a small container of burning sulphur lowered into the bottle and the bottle tightly stoppered. After exposure to the sulphur dioxide for about 15 minutes, the butter had developed a

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distinct pink color which became very pronounced on further exposure; in butter exposed for 19 days the pink color had penetrated to a depth of about 5 mm. It was noted that butter which had partially dried out by exposure to the air developed the pink color more quickly than freshly cut butter.

HISTORY OF THE BUTTER

Inquiry revealed that the butter developed the pink color in the refrigerator of a consumer; the machine used sulphur dioxide as a refrigerant. In the retail establishment that distributed it, the butter may have been held in a refrigerator using methyl chloride as a refrigerant. The butter was known to have been made with certified butter color.

After the development of the defect, prints of butter were held in the refrigerator of the consumer without an abnormal color developing; but later a pink color appeared in butter from two manufacturers when it was held in the refrigerator. One of the lots came from the manufacturer supplying the original butter and it definitely had not been in the refrigerator using methyl chloride.

CASE 2

Two one-pound prints of butter were sent to the laboratory by a distributor to whom the butter had been returned from the eastern market; the butter had developed the pink color in the refrigerator of a consumer. The prints were manufactured in two Iowa creameries.

DESCRIPTION OF THE DEFECT

One of the prints showed a great deal of pink color. Each of the ends was pink over most of the surface and wide, irregular, pink bands ran the length and width of each side near the edges while the extreme edges of the print and the centers of the sides were largely normal in color. Near the center of each side there were islands of pink color. The surface of the butter had apparently dried considerably since it showed salt crystals. Sections through the butter showed that the pink color involved only the surface and penetrated to a depth of about 1 mm.

The other print showed only one small pink area and this was on an end; it seemed to be related to a fold in the parchment. Sections through the print showed no pink color beneath the surface.

EXPERIMENTAL

A microbiological examination of the butter indicated that neither of the prints contained unusual numbers nor types of organisms.

Butter from each print was exposed to sulphur dioxide (obtained by burning sulphur) in a tightly closed bottle and a pink color quickly developed.

HISTORY OF THE BUTTER

Inquiry by the butter distributor revealed that certified butter color was used by the two plants supplying the butter. An examination, by a representative of the firm manufacturing the refrigerator in which the butter had developed the defect, showed that there was a leak in the gas line that permitted the escape of sulphur dioxide into the food compartment.

ACTION OF VARIOUS REAGENTS ON BUTTER COLORS

In order to study the action of various reagents on butter colors, four brands of color were selected. Three of these were marketed as certified butter color and the fourth as annatto color; the certified preparations gave tests for coal tar dyes while the annatto preparation did not. Each of the butter colors was worked into uncolored butter, both salted and unsalted, in an amount sufficient to give the butter a pronounced color. These samples, as well as the butter mentioned in cases 1 and 2 (which was reported to have been made with certified colors), were then treated with various reagents.

The treatment with a gas was effected by placing small samples of the butter in a large wide-mouthed bottle, filling the bottle with gas from a cylinder or generator, and then tightly closing the inlet and outlet tubes. The samples were treated with vapors or fumes by placing them, along with a small beaker of the reagent, in a large wide-mouthed bottle which was then tightly stoppered. Liquid and solid reagents were added directly to the butter after it had been placed on pieces of parchment in petri dishes.

RESULTS OF VARIOUS TREATMENTS

All of the butter samples colored with the certified colors became pink when exposed to sulphur dioxide or chlorine, or to bromine vapor while the butter containing the annatto color showed only a bleaching. None of the samples of butter showed any color change on exposure to methyl chloride, hydrogen sulphide or carbon dioxide. The fumes of hydrochloric or nitric acid caused the development of a distinct pink color in all the butter colored with certified preparations, but there was no change in the butter colored with annatto. The vapor of acetic acid effected no change in color in any of the samples, although the butter became very acid to the taste. Ammonia vapor caused a slight bleaching with all of the butter. Samples of butter which had become pink by exposure to hydrochloric acid bleached perceptibly when exposed to ammonia vapor but failed to lose the pink color entirely. When added directly to the butter, concentrated hydrochloric, nitric, sulphuric, phosphoric or lactic acid caused the pink color to develop in the samples containing certified colors, while the butter colored with the annatto preparation was not affected. Nitric acid apparently gave the most rapid and intense reactions with the certified colors and, accordingly, it was used later in testing various samples of butter for certified colors. Concentrated acetic acid or saturated aqueous solutions of boric, citric, tartaric and tannic acids did not produce color changes in any of the samples.

Powdered calcium hypochlorite and solutions of sodium hypochlorite, calcium hypochlorite and sodium sulphite were added directly to the various samples of butter but none of them caused any color change except for a bleaching in certain instances.

With various reagents the butter colored with certified colors showed the color change sooner when it was dried out on the surface than when it was freshly cut, and the salted butter apparently reacted more readily than the unsalted, although the difference was very slight.

ACTION OF VARIOUS REAGENTS ON COAL TAR DYES
COMMONLY USED IN CERTIFIED BUTTER COLORS

The coal tar dyes commonly used in the manufacture of certified butter colors were studied from the standpoint of the color changes induced by

the various reagents used in the studies on butter colors. Each of the dyes, yellow AB (Benzeneazo-B-naphthylamine) and yellow OB (Ortho-tolueneazo-B-naphthylamine) was worked into uncolored butter, in an amount sufficient to give the butter a distinct color, and the butter then treated with the reagents. In general, the reagents which produced a pink color in the butter containing certified butter color, also produced a pink color in the butter containing yellow AB or yellow OB, but with yellow AB the pink color developed more quickly and was much more intense than with yellow OB.

The studies on yellow AB and yellow OB were repeated, using dyes from another manufacturer, and the same results were secured.

ACTION OF NITRIC ACID ON BUTTER FROM VARIOUS SOURCES

Samples of butter sent to the Iowa Agricultural Experiment Station from various sources for analysis or scoring were examined by treating each with a drop of diluted nitric acid (1:1). Of the 66 samples studied, 33 (or 50 per cent) developed a distinct pink color, and this usually was evident in one hour. The other samples showed no significant color change.

DISCUSSION OF RESULTS

The development of a pink color when normal butter from the prints showing the defect was exposed to sulphur dioxide, together with the information that the butter showing the irregular color was held in refrigerators using sulphur dioxide as a refrigerant, suggests that the action of escaping gas was responsible for the color defect. This explanation is substantiated by the fact that, in the one case in which the refrigerator was examined, a leak which permitted gas to escape into the food compartment was found by a representative of the firm which had manufactured the refrigerator. Furthermore, all of the defective butter was known to have been made with certified butter color and experimental butter made with any one of the three certified colors studied became pink when exposed to sulphur dioxide.

It should be recognized, however, that there are other reagents that can be responsible for the development of a pink color in butter made with certified color. While most of these reagents are not likely to come in contact with butter, a pink color in butter that is not due to microorganisms should only be attributed to sulphur dioxide after a careful consideration of the conditions under which the defect developed.

The action of sulphur dioxide on butter made with certified color presents a situation that should be definitely recognized in connection with the use of this refrigerant.

SUMMARY

Print butter, showing areas of distinct pink color at the surface, was forwarded to the laboratory from two widely separated sources, with the request that the cause of the pink color be determined.

Microbiological studies indicated that the flora of the butter included neither unusual numbers nor types of organisms. When portions of the butter that were normal in appearance were exposed to sulphur dioxide, a pink color quickly developed. The butter from each source had been held in a mechanical refrigerator using sulphur dioxide as a refrigerant and, in

the one instance in which an examination was made, a leak permitting sulphur dioxide to escape into the food compartment was located by a representative of the firm manufacturing the refrigerator.

Butter from each of the sources developed a pink color when exposed to sulphur dioxide or chlorine, to bromine vapor, to the fumes of hydrochloric or nitric acid, or when concentrated hydrochloric, nitric, sulphuric, phosphoric, or lactic acid was placed on the butter. A number of other reagents failed to produce a pink color. The butter was reported to have been made with certified color. Three small lots of butter prepared in the laboratory with three certified colors reacted with various reagents in the way that the normal butter from the prints showing the pink color had reacted, but one lot made with annatto color did not develop a pink color with any of the reagents. Butter containing either yellow AB or yellow OB also developed a pink color when exposed to a number of reagents; with yellow AB the pink color developed more quickly and was much more pronounced than with the yellow OB.

Sixty-six samples of commercial butter from various sources were treated with diluted nitric acid (1:1) and 33 (or 50 per cent) of them became distinctly pink.

A STUDY ON THE USE OF ARSENICAL DUST FOR THE CONTROL OF JUNE BEETLES¹

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In June of 1932 the members of the Golf and Country Club at Hampton, Iowa, were greatly concerned over the appearance of large numbers of June beetles which were defoliating their oak shade trees. Spraying or dusting seemed out of the question, partly due to the scarcity of experimental data on the effectiveness of these measures and partly due to the unavailability of the necessary spraying and dusting equipment. The members of the Club, however, engaged a local airplane and urged the writers to coöperate with them in conducting an experiment in airplane dusting.

Shortly after daybreak on June 14, while the leaves were still wet with dew, dusting operations were started. After a small area had been dusted it was observed that the rate of application was much too light. On the next trip, which was to have doubled the rate of application over the same area, the plane was badly damaged in making a forced landing, and dusting was necessarily discontinued.

With only a small part of the grove very lightly dusted, it appeared at the time that the experiment would be a complete failure. However, three days after treatment many dead beetles were found on the ground under the trees. The beetles continued to die by the thousands for three or four days, after which time no additional dead beetles were observed. With this encouragement, the writers decided to conduct laboratory tests to secure additional data on the value of calcium arsenate dust as a means of controlling June beetles.

METHODS

In each experiment, beetles that had been collected from oak trees were confined in screen covered cages which were placed over trays of soil. Just after sundown fresh oak leaves were gathered and lightly sprayed with water to give conditions somewhat comparable to foliage wet by fog or dew. These leaves were then spread on the ground and dusted with poison by means of a hand duster. The dust mixture used in the airplane test and in all of the laboratory experiments consisted of 40 per cent calcium arsenate and 60 per cent bentonite. (Bentonite was selected as a diluent because of its adherence properties). Dusted leaves were placed in the cages and the beetles were permitted to feed at will until morning.

No mathematical basis for calculating or regulating the exact amount of arsenic deposited on each leaf was devised. However, the poison was applied to the foliage at three distinct rates of application: (1) leaves dusted until nearly white (heavy); (2) leaves dusted until only a film of the arsenate appeared on the surface (light); (3) leaves dusted to an intermediate degree (moderate).

¹ Journal Paper No. B113 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 128.

One-half of the beetles receiving poisoned foliage were given treated leaves on two successive nights, whereas the remaining half received poisoned foliage only during one night. Fresh untreated leaves were provided on the nights when treated leaves were not furnished in the cages. Duplicate series were run and, unless otherwise stated, 50 beetles were used in each test. Each experiment was accompanied by four checks, two of which received fresh, unpoisoned food each night and two of which received no food on nights when the experimental cages received dusted foliage. The soil was sifted daily to determine the number of dead beetles. An individual that was able to move its legs was considered to be alive. The sexes were caged and counted separately, because of the obvious difference in their death rates and an apparent difference in their susceptibility to poisoning. *Phyllophaga hirticula* (Knoch) was used in experiments I, II, III, and V, *P. rugosa* (Mels.) in experiment IV.

EXPERIMENTS

Four parallel experiments were made to determine the effects of various weather conditions upon the feeding habits and mortality of beetles given poisoned leaves. As the results appear significantly different, the conditions for each experiment will be discussed separately.

Experiment I. The beetle in this experiment received poisoned foliage on June 23, and those which were to receive a second treatment were given poisoned foliage on June 24 also. The cages were kept in a screened insectary where they were exposed to approximately out-of-door temperatures. The minimum temperatures on these two nights were 61° F. and 69° F., respectively. Under these conditions the beetles were active early in the evening and moderate feeding occurred. The morning after the first treatment it was observed that the margins of the leaves were almost free from poison, and, as the beetles normally eat along the edges of the leaves a high mortality was not to be expected. This proved to be true. (Experiment I, table 1).

Experiment II. This experiment was started June 30 under adverse weather conditions. The minimum temperatures for June 30 (58° F.) and July 1 (50° F.) were so low that the beetles were sluggish and very little feeding occurred. A heavy rain during the second night of the experiment washed much of the poison from the leaves so that the beetles consumed very little poisoned foliage. As a result there was an appreciable lowering of the number of beetles killed. (Experiment II, table 1).

Experiment III. On July 9 a third set of experiments was started, and to overcome the prevailing low temperatures, these beetles were placed in a warm room. Although the temperature of this room dropped from 75° F. to 65° F. during the night, the beetles were active and fed normally. The beetles became active early in the evening, but those given poisoned leaves ate very little, while those feeding upon unpoisoned foliage fed ravenously. This difference in the amount of treated and untreated foliage consumed was observed also in experiments I and IV. (Experiment III, table 1).

Experiment IV. On July 11, a series of *P. rugosa* was given the same treatment as the *P. hirticula* in experiment III. It appears that the two

TABLE 1. A summary showing the percentage of dead beetles 72 hours after treatment of foliage with calcium arsenate

Treatment	Female beetles					Male beetles				
	Experiment No.				Weighted mean	Control $\frac{x-y}{x}(100)$	Experiment No.			Weighted mean
	I	II	III	IV			I	II	III	
No. of beetles in each lot	50	50	50	25			50	50	40	
Heavy 2 nights	79	60	92	100			100	94	98	
Heavy 2 nights	85	70	98	100			98	86	100	
Average	82	65	95	100	84	82	99	90	99	96
Heavy 1 night	73	44	96	100			93	86	96	
Heavy 1 night	85	34	82	84			100	80	92	
Average	79	39	89	92	72	68	97	83	94	91
Moderate 2 nights	88	20	88	84			95	66	92	
Moderate 2 nights	52	12	66	88			66	78	100	
Average	70	16	77	86	59	53	82	72	96	82
Moderate 1 night		22	72	64			84	62	100	
Moderate 1 night		48	56	72			94	82	88	
Average	35	64	68		53	47	89	72	94	84
Light 2 nights		20	86							
Light 2 nights		18	40							
Average		19	63		41	33				
Light 1 night	44	12	60	72						
Light 1 night	36	16	22							
Average	40	14	41		37	29				
Check no feed	0	14	8	32				64	46	
Check on feed	4	16	4					76	36	
Average	2	15	6	32	11			70	41	37
Check fed	0	10	10	36						
Check fed	8	22	6				23	72	38	
Average	4	16	8	36	13		31	68	35	45

species are about equally susceptible to calcium arsenate poisoning. (Experiment IV, table 1).

Experiment V. Twenty-five *P. hirticula* females were rolled in calcium arsenate dust until their bodies were covered with the poison. They were then confined in a cage without food. At the end of the third day 16 per cent of the treated beetles and 20 per cent of the check beetles were dead.

DISCUSSION OF DATA

The data for the third day were selected for presentation, largely because of the rising death rate in the male checks. Had the data for the fifth or sixth day been chosen for presentation, the mortality of the female beetles would have been increased appreciably, especially in the moderate and light treatments; for example, in experiment I on the sixth day the percentage of beetles killed by the various treatments were, 99, 91, 88, 82, and 10 respectively, instead of 82, 79, 70, 40, and 2 as given in table 1 (third day). It should be noted that these tests were conducted late in the flight season and the high death rate of the males was due somewhat to their advanced age.

The formula, $\frac{x-y}{x} (100) = \text{percentage of control}$ (where $x = \text{number live in checks and } y = \text{number live in experiments}$) was applied to the data in order that the results for the two sexes might be more easily compared. The use of this formula did not materially change the relationship existing between the percentage killed in the various experiments, and the death rate of the males continued to be higher than that of the females.

The application of the formula to the data of the third day unduly reduced the values for the heavy treatments because most of these beetles had died on the first and second days when the number dead in the checks was very low. Also, the high mortality of the checks in experiment IV gave an unjustifiably low value to y and therefore, further reduced the apparent control values.

Great differences were observed in the daily death rate of beetles receiving leaves which were coated with varied amounts of the arsenical. The heavy applications killed in 24 to 48 hours, whereas the lighter treatments did not give high kills until 48 to 96 hours after the beetles had fed upon the poisoned foliage. In fact, in all experiments the moderate and light applications gave high mortalities as late as 5 or 6 days after treatment. Figure 1 illustrates graphically the percentage of dead beetles occurring 24, 48, 72, and 96 hours after treatment for both male and female beetles. The data for these curves were taken from experiment III, because this experiment was conducted under reasonably favorable conditions. The same data taken from the other experiments are so similar that repetition of the graphs seems unwarranted.

When it was observed that the beetles fed sparingly upon poisoned foliage the question of a possible increase in mortality due to voluntary starvation arose. This problem was disposed of favorably when the checks, which received no food for two days, showed no appreciable mortality over those fed every day. The reduced feeding on poisoned foliage was probably due to the first effects of the poison causing a cessation of feeding.

Since very little feeding occurred on leaves receiving heavy applications of dust, it was suspected that the beetles might have received a lethal

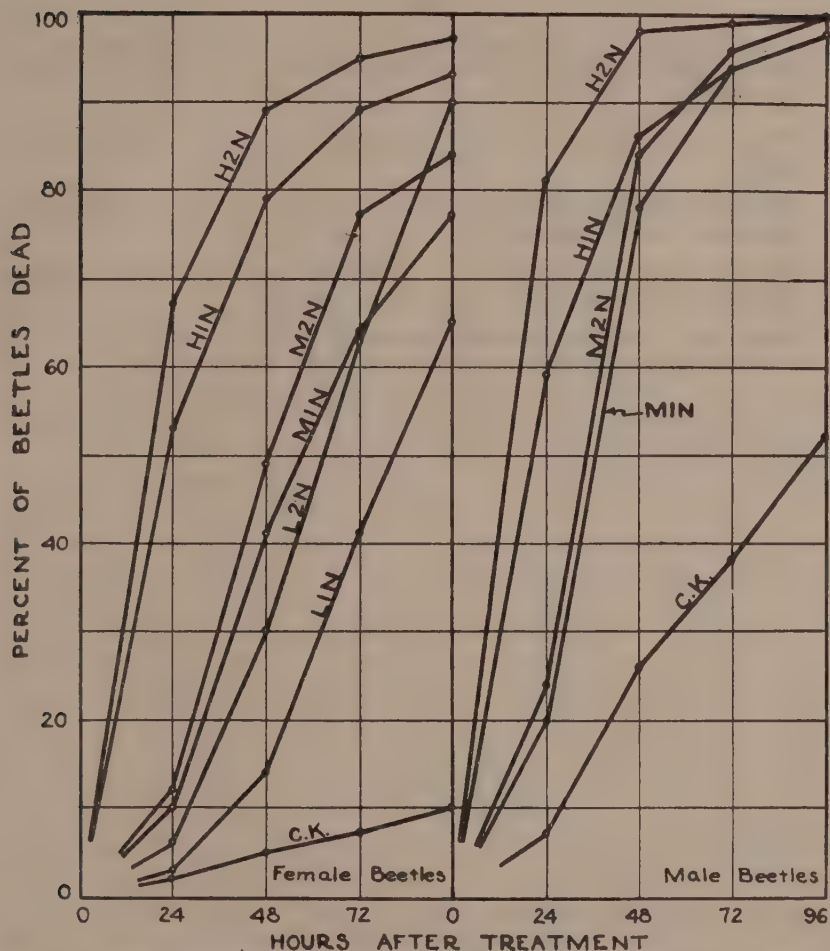


Fig. 1. A graph illustrating the percentage of beetles dead, 24, 48, 72, and 96 hours after treatment. The quantity of arsenic and the number of nights poisoned foliage was offered are indicated as follows: H1N = Heavy one night, H2N = Heavy two nights, M1N = Moderate one night, M2N = Moderate two nights, L1N = Light one night, L2N = Light two nights, and CK = checks.

dose of poison by cleaning their dust-covered appendages with their mouthparts. The results of experiment V proved rather conclusively that beetles in experiments I, II, III, and IV were killed by eating poisoned foliage. In fact the writers saw no evidence of the so-called "cleaning up" habit displayed by some insects.

In all of the experiments, except number II, good results were secured by moderate and heavy applications of dust and fair results were secured from light applications. If this data can be substantiated by further experiments, dusting with calcium arsenate can be recommended as an efficient method of preventing defoliation by June beetles.

SUMMARY

1. It was evident that the beetles were killed by eating poisoned food and not by ingesting free dust from the surface of the leaves or from their appendages.
2. Males appeared to be more susceptible to arsenical poisoning than females.
3. When the temperature was too low to induce normal feeding, good results could not be secured.
4. Moderate and heavy applications of calcium arsenate killed from 65 to 100 per cent of the beetles in less than 72 hours.
5. There was a direct correlation between the rate of application of the dust and the time required to kill the beetles.
6. Undusted foliage was readily consumed, whereas, poisoned leaves were eaten only sparingly.

OBSERVATIONS ON THE WINTER SURVIVAL OF PLANT LICE IN IOWA (HOMOPTERA-APHIIDAE)¹

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A cursory review of entomological literature reveals that plant lice are thought to overwinter principally in the egg stage in the northern part of the United States. While studying the hibernation habits of hemipterous insects, the writers obtained data showing that in Iowa certain species of plant lice may overwinter in the nymphal and adult stages of apterous and alate viviparae as well as in the egg stage.

Knowledge of the fact that a certain percentage of the summer forms of some plant lice survive the inclement season in the more northern states, may add considerably to our understanding of the behavior of this group of insects. It is not unlikely that spring and early summer injury may be more or less correlated with winter survival of viviparous forms. Furthermore, the economic importance of this phenomenon may be greatly augmented by the fact that certain species of the family Aphididae are known to be the natural agencies concerned in the dissemination of numerous virus diseases of plants. Since all stages of viviparae of some species are able to survive the winter and then to continue their activities in the spring, it is possible that they may serve as overwintering reservoirs for plant viruses. Interest in the latter possibility was further stimulated because only recently (Drake, Harris, Tate, 1932) plant lice have been shown to be associated with the dissemination and perpetuation of yellow dwarf, a serious virus disease of cultivated onions in eastern Iowa.

Records of the winter survival of plant lice in the southern states are not uncommon—especially in the Carolinas, Tennessee, Oklahoma and Texas where the mean normal temperature during the winter months is approximately 40° F. or higher and the minimum temperature seldom approaches 0° F. This survival, however, is probably not “true” hibernation but is in reality a period of suspended activity due to low temperatures. Plant lice found in such areas in the spring are probably the offspring of more or less continuous viviparous reproduction and not the individuals present the previous fall.

Wadley (1931) reported that *Toxoptera graminum* Rondani was apparently able to survive the cold seasons where minimum temperatures were somewhat above 0° F. and temperatures were often high enough to permit some development. According to Kelly (1917), *T. graminum* may survive in Kansas, especially during the mild winters. Matheson (1919) collected viviparous females of the apple grain aphid, *Rhopalosiphum prunifoliae* (Fitch), near the bases of their summer host plants but did not determine whether these forms could live through the winter and then continue their normal activities in the spring. Baker and Turner (1916)

¹ Journal Paper No. B114 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 135.

collected small numbers of *Aphis pomi* DeGeer in Virginia which had been subject to -6°F. on one occasion in January. No records of survival as far north as central Iowa or Nebraska have been found in the literature by the writers.

For collecting aphids in Iowa during the winter season, a modified Berlese funnel was found to be quite suitable. A large cone-shaped funnel 20 inches in diameter at the top and tapering to one inch at the bottom with a circular wire screen of a sufficient diameter to set about four inches below the top of the cone was suspended in a frame so that a pint milk bottle could be readily placed under it. Materials such as leaf mold, moss, sod and the like were brought in from the field, and spread more or less uniformly over the screen. Anything crawling or falling through the screen was directed by the funnel into the bottle below, which was partially filled with alcohol, except when living material was desired. Certain of the more active insects such as Diptera, Hymenoptera, and Neuroptera not infrequently escaped from the debris, and it seems highly probable that some of the aphids either perished in handling the material or escaped in the laboratory.

The average temperature (figure 1) at Ames, Iowa, during October, November and December (1932) was several degrees below normal (58-year mean). Conversely, January 1933 was the warmest January during the 58 years that weather records have been recorded at this station, whereas, February and March were only slightly above normal. The maxima and minima as shown in the graph, figure 1, represent both the highest and lowest temperatures recorded at any time during the month concerned—thus covering only a one-day reading—and represent the actual extremes. The lowest temperature recorded during the winter was -23°F. (Feb. 9). On February 25, two alate females of *Rhopalosiphum prunifoliae* (Fitch) were taken from bluegrass which was collected in an open field near Ames. The ground at the time had only traces of snow on it so that the aphids had been exposed openly to the cold for some time. Likewise, at Ottumwa, Iowa, on December 26 (1932) aphids representing three different genera—namely, *Myzus*, *Aphis*, and *Macrosiphum*—were collected from moss after having been subjected to a temperature of -19°F. on December 16. Although a covering of snow, no doubt, affords considerable protection to aphids and other insects during cold periods all of the specimens taken by the writers were from exposed areas where no snow-cover occurred at the time collections were made. The apple grain aphid, *Rhopalosiphum prunifoliae* (Fitch), was found more frequently than any other species. It occurred most commonly in *Thuridium delicatulum*, (L.) Nutt. a moss, as well as in bluegrass sod.

Table 1 includes a list of the aphids² taken together with the locality and the material in which they were found.

In several instances it was impossible to determine the forms further than the genus because only nymphs, one or two adults, or specimens mutilated in handling were secured. Some idea concerning the number of plant lice that may survive in a given area can be obtained from the following records taken at Ottumwa, Iowa. An area containing approximately four square feet of moss was marked off, loosened about one inch below the ground-line, rolled up in a carpet-like manner, and then placed in collect-

² Aphidiidae determined by Dr. F. C. Hottes.

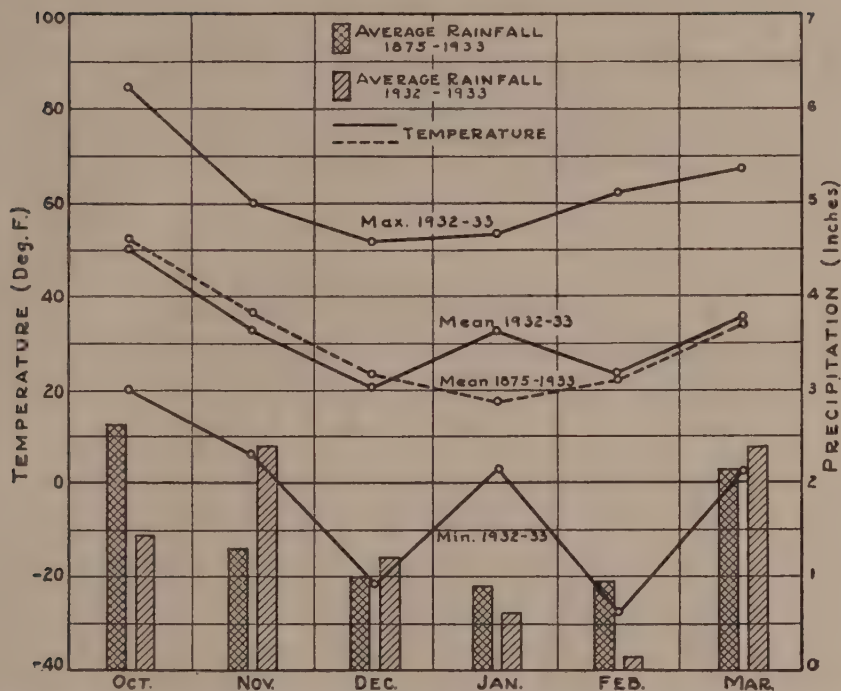


Fig. 1. Graph showing maximum, minimum and mean monthly temperatures for the winter of 1932-33, and the mean temperature from 1875 to 1933, as recorded by the U. S. Weather Bureau observation station at Ames, Iowa. Rainfall is shown by rectangular bars.

ing funnels with the plant side resting upon the screen. From this material one alate and two apterous viviparae and four nymphs were obtained. Bluegrass sod is also a suitable medium from which to obtain overwintering individuals, for in several instances from three to five plant lice were collected in an area containing approximately three square feet on the college grounds at Ames. Based upon these plots the number of aphids that survive in favorable overwintering areas may be well above seventy-five thousand to the acre. Winter survival of summer forms is without question greatly influenced by the fall and winter aspect of the surrounding vegetation.

It has been observed by the writers that mature individuals, collected in the field during the early spring before the temperatures were sufficiently high to permit normal activity, will feed and begin viviparous reproduction when they are subjected to favorable conditions. On April 5 (1933) when the outdoor temperature was about freezing, several viviparous adults of *Rhopalosiphum prunifoliae* (Fitch) were collected from bluegrass sod and confined on growing plants in the greenhouse. Within a few hours these females began parthenogenetic reproduction and continued their life processes in a normal manner.

TABLE I. *Showing species of aphids collected, date of collection, stage of development, habitat and locality*

Date collected	No. collected	Stage*	Genus and species	Habitat	Locality
Nov. 24	1	Nymph	Macrosiphum sp.	Moss	Ottumwa, Ia.
Nov. 28	3	Adults	Rhopalosiphum prunifoliae (Fitch)	Moss	Ames, Ia.
Nov. 28	2	Nymphs	" "	Moss	Ames, Ia.
Dec. 26	5	Nymphs	Myzus sp.	Moss	Ottumwa, Ia.
Dec. 26	1	Nymph	Sub. fam. Eriosomatinae	Moss	Ottumwa, Ia.
Dec. 26	1	Adult (alate)	Rhopalosiphum prunifoliae (Fitch)	Moss	Ottumwa, Ia.
Dec. 26	4	Nymphs	Macrosiphum sp.	Moss	Ottumwa, Ia.
Dec. 26	4	Adults (alate)	Macrosiphum pisi (Kalt)	Moss	Ottumwa, Ia.
Dec. 26	5	Nymphs	Aphis sp.	Moss	Ottumwa, Ia.
Feb. 20	1	Adult (apterous)	Rhopalosiphum prunifoliae (Fitch)	Moss	Ottumwa, Ia.
Feb. 25	2	Adults (alate)	" "	Bluegrass	Ames, Ia.
March 16	1	Adult (alate)	" "	" "	Ames, Ia.
March 20	1	Adult (alate)	" "	Moss	Ottumwa, Ia.
March 20	2	Adults (apterous)	" "	Moss	Ottumwa, Ia.
March 20	4	Nymphs	" "	Moss	Ottumwa, Ia.
April 5	8	Adults (apterous)	" "	Bluegrass	Ames, Ia.

* All specimens were viviparae.

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TIME OF EXPOSURE AND TEMPERATURE AS LETHAL FACTORS IN THE DEATH OF THE OOCYSTS OF *EIMERIA MIYAIRII*, A COCCIDIUM OF THE RAT

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Pérard (1925) first demonstrated that the oocysts of *Eimeria perforans* and *E. stiedae* of the rabbit are relatively susceptible to heat as compared with the high degree of resistance they offer to ordinary chemical disinfectants. The next workers to study the lethal effects of heat upon coccidia were Becker and Crouch (1931), who found that the oocysts of both *Eimeria magna* and *E. perforans*, in a two per cent aqueous solution of potassium dichromate, were killed by a ten minute exposure to a temperature of 51° C. More recently, Fish (1932) has made a rather extensive study of the lethal effects of various temperatures and chemical solutions acting for varying lengths of time upon the oocysts of *Eimeria tenella* of the chicken. It is of special interest in the present discussion that he noted the death of both the unsporulated and sporulated forms at a temperature of approximately 53° C. for an exposure interval of 10 minutes.

The object of the present study was to establish the thermal death point of the sporulated and unsporulated oocysts of *Eimeria miyairii* (i.e., the lowest temperature which will kill 100 per cent of the organisms during a 10 minute exposure), and to determine the relation of time and temperature to mortality.

The technique employed differed somewhat from that of previous experimenters. The unsporulated oocysts were taken directly from the caecum of the rat on the eighth day after the date of infection with the sporulated material. At this time numerous unsporulated oocysts were emerging from the intestinal epithelium and so became free in the lumen. When the oocysts appear in the pellets after passing through the whole length of the intestine, they often undergo considerable mortality, perhaps due to desiccation. But when they are taken directly from the caecum, 98-100 per cent of them complete their development in appropriate media, so that no mortality factor in the controls needs to be taken into consideration in calculating the percentage of death due to experimental conditions.

An electrically controlled water bath was constructed to hold a constant temperature within approximately 0.2° C. The content of the caecum was first mixed with a 2.0 per cent potassium dichromate solution. This material was centrifuged, then mixed with distilled water, centrifuged again, and the process repeated three times. After the last washing the material was mixed with saturated salt solution, and the oocysts floated by centrifuging. The water bath was adjusted to a certain constant trial temperature. Sterile specimen vials containing 2 cc. of sterile distilled water were suspended in the bath. A 4 mm. horizontal platinum loop was used in transferring a 0.01 cc. suspension of oocysts from the surface of the salt to one of the vials in the bath. At the moment the loop was immersed in the vial a stop-watch in the left hand was punched. At the end

TABLE 1. *The effect of heat on the development of the unsporulated oocysts of Eimeria miyaii*

Temperature degrees C.	Time of exposure	Percentage of development (sporulation)
41	24 hrs.	0.0
	21 hrs.	1.0
	18 hrs.	10.0
	15 hrs.	34.0
	12 hrs.	66.0
	9 hrs.	80.0
	6 hrs.	88.0
	3 hrs.	97.0
	2 hrs.	10.0
45	2.5 hrs.	0.1
	3 hrs.	0.0
47	20 min.	60.0
	25 min.	15.0
	28 min.	9.0
	31 min.	0.0
48	13 min.	18.0
	14 min.	10.0
	16 min.	0.0
49	5 min.	4.0
	6 min.	2.0
	7 min.	0.0
50	3 min.	3.0
	4 min.	0.0
51	1 min.	1.0
	2 min.	0.0
52	0.25 min.	40.0
	0.50 min.	1.0
	1 min.	0.0
53	0.083 min.	88.0
	0.16 min.	60.0
	0.25 min.	0.0
	0.50 min.	0.0

of the trial period of exposure the vial was removed swiftly and plunged into cold water. The vial content of fresh undeveloped material was plugged with cotton and set aside for 72 hours to permit sporulation at room temperature. At the end of this time, a specimen was drawn off from the bottom of the vial for microscopic examination. In the case of the unsporulated material, the criterion of death was the failure of the organisms to form spores within the cyst. When organisms failed to initiate sporulation within 72 hours, a longer incubation proved to be of no further value.

Sporulated oocysts for the temperature tests were obtained by "cultivating" the unsporulated forms from the rat cecum in 2.0 per cent potassium dichromate. The sporulated material was concentrated and subjected to a trial temperature in the manner just described for the unsporulated forms. After the vial was plunged into cold water, the content was fed to a young rat known never to have become infected previously, and hence to be very susceptible. After seven to eight days, pellets were expressed from the rectum of the rat for microscopic examination. The continued absence of unsporulated oocysts from the feces was the criterion for the death of the sporulated oocysts; and, conversely, the presence of unsporulated oocysts was considered to be indicative of survival at the trial temperature.

TABLE 2. *The effect of various temperatures and times of exposure on the infectivity of the sporulated oocysts of Eimeria miyairii for the white rat*

Test rat No.	Temperature degrees C.	Time of exposure in minutes	Result
4	48	10	+
8	48	10	+
3	49	10	—*
13	49	10	+
19	49	10	— [†]
2	50	10	—
5	50	10	—
14	50	10	—
1	51	10	—
6	51	10	—
23	51	5	—
24	51	4	—
25	51	3	—
7	52	10	—
15	52	4	—
20	52	1	+
21	52	2	—
22	52	3	—
16	55	1	—
17	55	2	—
18	55	3	—

In the technique employed by Fish, 0.5 cc. of a suspension of the oocysts was introduced into an exposure vial containing 4.5 cc. of 2 per cent potassium dichromate solution. This procedure would increase the volume of liquid in the vial by one tenth, lower its temperature slightly and introduce a small source of error. In the technique employed in this experiment, 0.01 cc. of film of the suspension of coccidia on a loop was introduced into 2 cc. volume of liquid in the exposure vial. This procedure increases the volume of liquid in the vial by only one two-hundredth, and introduces but a negligible error on account of the cooling. The flotation method makes it possible to secure enormous numbers of the organisms, and the film on the platinum loop was usually clouded with them. A loopful on a slide would darken the field of the microscope. Likewise, flotation frees the coccidia from most of the organic matter, so that the use of a dichromate solution in the exposure vial to prevent bacterial action is unnecessary. Fouling of these culture tubes did not occur. Controls were maintained in all experiments on the unsporulated forms, and they always showed an incidence of development of from 98 to 100 per cent.

The results of the experiments are shown in tabular form in tables 1 and 2, and graphically in figure 1. It was found that the unsporulated oocysts are killed in 15 seconds at a temperature of 53° C. and in 24 hours at a temperature of 41° C. Thus, the lethal effects of heat accelerate rapidly with the increase of temperatures between these two points. The thermal death point, i.e., the lowest temperature at which death occurs as the result of a ten minute exposure, is between 48 and 49° C., because death was noted at 48° in 16 minutes and at 49° C. in 7 minutes. It is shown at 48.5° C. in the curve (fig. 1, B). The sporulated oocysts were all

* One positive and two negative results for this time and temperature indicate that 49° C. is approximately the thermal death point.

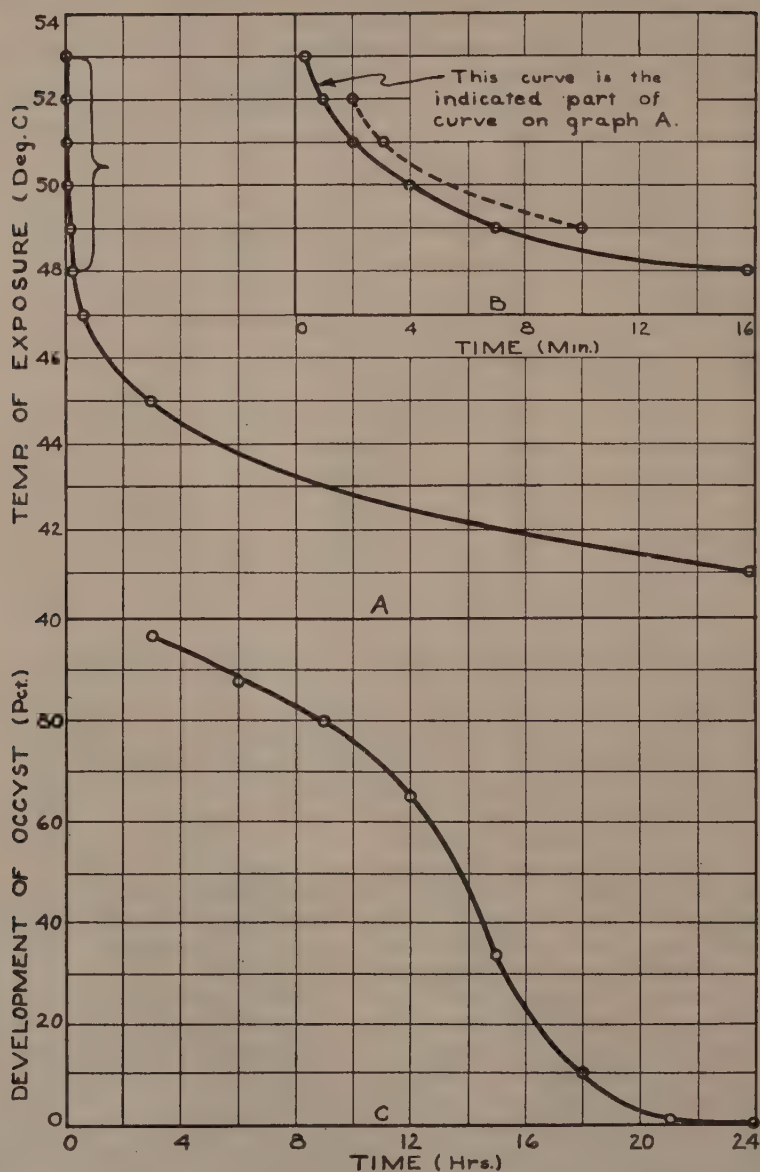


Fig. 1. A. This graph shows the time in hours required to kill the unsporulated oocysts of *Eimeria miyairii* at different selected temperatures.

B. (Inset). The black line shows the time in minutes required to kill the unsporulated oocysts of *Eimeria miyairii* at the higher temperatures represented in A. The broken line, based upon the results of three test temperatures, shows the time in minutes required to kill the sporulated oocysts.

C. This graph depicts the percentage of development after the unsporulated oocysts have been kept for various lengths of time at 41°C.

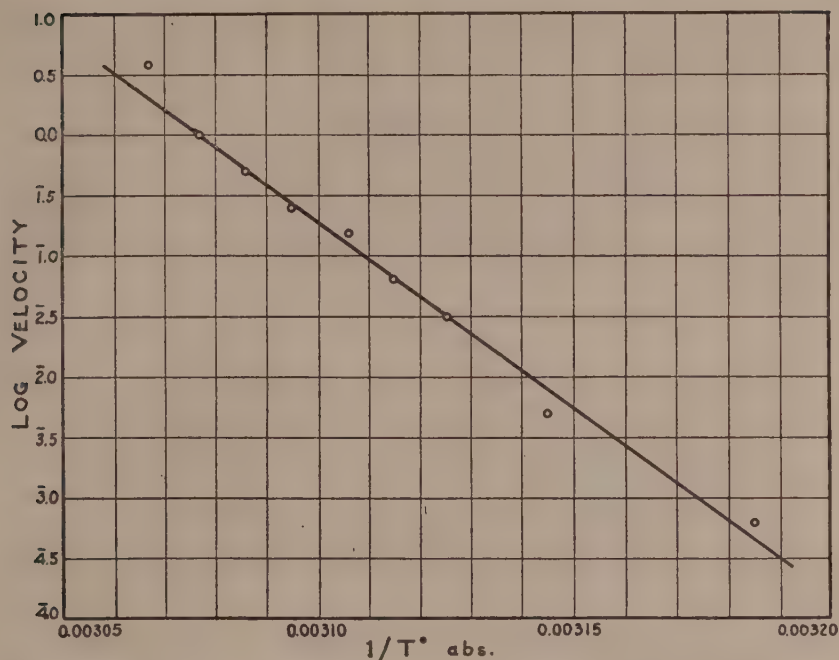


Fig. 2. Log velocity ($= \log 1/\text{time in minutes}$) plotted against $1/\text{abs. temp.}$

killed at 49°C. in 10 minutes in two tests out of three, so this temperature must be very near their thermal death point. The fact that the death points for the sporulated oocysts are shown from a half to a full degree above the same for the unsporulated oocysts at the three test temperatures does not necessarily indicate any important difference in their tolerance for heat, because it must be remembered that thousands of the heat-tested oocysts were fed to the rats, and if only one or several of each test had survived the trial temperature the rats would have become infected and the results would have been recorded as positive.

Since the unsporulated oocysts die very slowly at 41°C. , an experiment was performed to determine the percentage of survival of the oocysts for varying lengths of time (Fig. 1, C). At this temperature all were killed in 24 hours, about 65 per cent survived after an exposure of 12 hours, and practically all survived after an exposure of 3 hours. The curve shown is somewhat S-shaped.

It was suggested that the van't Hoff-Arrhenius equation,

$$K_2 = K_1 e^{\frac{\mu}{2} \left(\frac{1}{T_1} - \frac{1}{T_2} \right)}$$

be applied to the data for the time and temperature required to kill the unsporulated oocysts, and the value of the critical thermal increment (μ of Arrhenius, 1889) be determined. First, the values for the reciprocals of the absolute temperatures were obtained, and then the logs of the reciprocals of the various lengths of time (in minutes) required to kill at these temperatures. Then the values in the first set were plotted against the cor-

responding ones in the second (Fig. 2). It may be noted that of the nine points in the graph, six lie almost on a straight line, while the other three are not far removed.

When the velocity constants (K_1 , K_2) and absolute temperatures (T_1 , T_2) corresponding to several of the points nearest the line were substituted in the previously stated formula, μ values of from 140,000 to 146,000 were obtained. It should be stated, however, that the experiment was not planned with this analysis in mind. It is possible that subsequent work will show that some other point, such as the half-kill or three-fourths-kill, is a more accurate criterion for the time and temperature effects than total kill.

CONCLUSIONS

1. The thermal death point (10 min. exposure) for the sporulated and unsporulated oocysts of *Eimeria miyairii* is approximately 49° C.,—apparently a fraction of a degree less for the unsporulated forms.
2. The unsporulated forms are killed in 15 seconds at 53° C., and in 24 hours at 41° C.
3. The sporulated forms perish in 2 minutes at 52° C.
4. The critical thermal increment (μ) for the death of the unsporulated oocysts, as computed by the van't Hoff-Arrhenius equation, is about 140,000.

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